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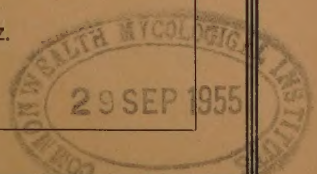

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(AGRICULTURAL RESEARCH SECTION)

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NOTICES

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AVAILABILITY OF IODATE IODINE TO SHEEP

By EVAN WRIGHT and E. D. ANDREWS, Animal Research Station, Department of Agriculture, Wallaceville

(Received for publication, 4 May 1955)

Summary

Experiments with radioactive iodine (^{131}I) given orally to six sheep as potassium iodate and as potassium iodide, showed that for four of the animals the amounts of iodine accumulated by thyroid glands from iodate and from iodide were comparable. In the remaining two animals maximal uptake from iodate was much lower than that from iodide. Nevertheless, in all six sheep the amounts of iodate iodine accumulated by the thyroid glands were sufficient to justify the conclusion that potassium iodate is a suitable source of iodine for sheep.

INTRODUCTION

Goitre occurs among farmstock, particularly in new-born lambs, in many parts of New Zealand. Although the aetiology of the disease is not yet clear it seems that a deficiency of iodine is at least one important cause (Hopkirk *et al.* 1930). Prenatal treatment with potassium iodide has prevented goitre in lambs from ewes on pasture and on thousand-headed kale (*Brassica oleracea* var. *acephala*) (Sinclair and Andrews, 1954).

Because topdressing with iodized fertilizers does not increase the iodine content of feeds for satisfactory lengths of time, extra iodine for stock is usually provided by salt licks containing 10 oz. of potassium iodide to the ton of lick (Waters, 1939). Under open-air conditions, iodine losses from salt licks containing potassium iodide can be serious. For instance Davidson and Watson (1948) found that after two months virtually all the iodine was lost from mixtures of salt and potassium iodide.

Methods of stabilizing potassium iodide in salt have been proposed. Johnson and Frederick (1940) suggested the use of calcium stearate, while Clifcorn, Greim, and Hart (1939) have recommended additions of sodium thiosulphate, and sodium carbonate. Nevertheless, Davidson and Watson (1948) could not demonstrate that these agents reduced iodine losses under open-air conditions.

Alternatives to potassium iodide have been suggested. Of these, potassium iodate has the advantages of being easily obtainable, and comparable in price with potassium iodide. Davidson, Finlayson, and Watson (1951) have shown that potassium iodate in salt blocks is stable when tested under practical feeding conditions. These investigators also concluded that iodine fed as iodate was physiologically available to sheep and swine because it was found in the urine as potassium iodide. According to Scrimshaw *et al.* (1953) potassium iodate and potassium iodide proved about equally effective in the treatment of endemic goitre and in raising the average level of serum protein-bound iodine in schoolchildren.

Using iodate labelled with radioactive iodine, it has been shown that iodate iodine is available to the thyroid glands of rats (Leblond and Süe, 1941) and humans (Murray and Pochin, 1951). Because of the probable future importance of iodate as a source of iodine to stock, it is desirable to obtain similar direct information concerning the accumulation of iodine from iodate by the thyroid glands of sheep. The present paper compares the uptake by sheep thyroids of radioactive iodine (^{131}I) from potassium iodate with that from potassium iodide.

EXPERIMENTAL

Methods

The ^{131}I was obtained from the Radiochemical Centre, Amersham, United Kingdom, as carrier-free sodium iodide or as potassium iodate with a specific activity of 1 mc. per mg. of iodine. Potassium iodide carrier was added to the ^{131}I—sodium iodide at the rate of 1 mg. iodine per mc. so that the preparation was, in effect, labelled potassium iodide.

Although administration of radio-iodine to sheep has been described, no information concerning minimal activity levels likely to cause radiation damage could be found. Talmage, Monroe, and Comar (1954) injected ^{131}I into sheep but did not state the activity of their preparation. Care (1954, unpublished report) dosed sheep orally with 100μc. ^{131}I but this rate was apparently arbitrarily chosen. For human beings, ^{131}I dosages have ranged from 5μc. (Starr and Liebhold-Schueck, 1953), through many intermediate values, to 150 μc. (Freedberg, Ureles, and Hertz, 1949), the most usual dose being 100μc. If the human thyroid is taken as weighing approximately 30 g. (Johnston and Willis, 1946), this corresponds to a dose rate of about 3μc./g. of thyroid tissue. Skanse (1948) showed that, for chickens, ^{131}I dosed at a level of 100 μc./g. of thyroid tissue had no apparent effect on thyroid growth. In the present work an activity of 25 μc. per dose was chosen. This would correspond to a dose rate of approximately 10 μc./g. of thyroid tissue since other work (Andrews and Whitten, 1954, unpublished) has shown that, for sheep of an age, breed, and previous history comparable to those used in the present investigation, weights of thyroid glands were of the order of 2.5 g. It was considered that at the level of activity chosen the dose would be high enough to ensure sufficient sensitivity with the methods of measurement available, and at the same time risk of radiation damage to the thyroid glands should be low.

Radio-iodine was administered by stomach tube, care being taken that no activity was left in the mouth or oesophagus to interfere with measurement of thyroid radiation.

The animals used were six one-year-old Romney wethers. They were held on the same paddock of perennial ryegrass and white clover for the duration of the investigation. For each separate experiment the sheep were brought indoors and held without food for 24 hours before administration of the ^{131}I compound and for 48 hours thereafter. For the rest of each indoor experimental period they received hay and concentrates *ad lib.*

Three experiments were carried out with the same animals at intervals of approximately one month to allow thyroid radio-activity to fall to a low level. The first and second experiments were made with labelled potassium iodide and potassium iodate respectively. In the third experiment a second dose of potassium iodide was given. The object of this was to check for possible changes in iodine uptake which might arise as a result of radiation damage from previous doses, or from variations in the nature of the pasture during the investigation.

Measurement

The animals were restrained in a special crush with their thyroids positioned at 33 cm. from a gamma-ray counter with a lead-filled copper cathode. The sensitivity of the counter was approximately 10 cps. per 25 μc. of ^{131}I at 33 cm. All counts including background counts were made over a period of five minutes.

The standard used was a simple thyroid phantom similar to that of Hine *et al.* (1953). This consisted of a 50 ml. standard flask containing 25μc. of radio-iodine solution in a 1,000 ml. beaker of water and positioned 1 cm. from the side nearest the counter. Measurements for each of the six animals were made at the intervals shown in Fig. 1.

RESULTS AND DISCUSSION

In Fig. 1 results are shown as percentages of the count obtained for the thyroid phantom. Because some absorption of radiation by glassware used in the latter was inevitable, figures are regarded as only approximating to the true percentages of iodine taken up by the glands. Since, however, the error is the same for all measurements, results from the three separate experiments may be compared.

Variability Between Sheep in Iodine Uptake from Potassium Iodide

Comparison of results obtained for the different sheep show that maximal uptake of iodide iodine varied from about 50% (Sheep 4) to 70–80% (e.g. Sheep 2). It is not known whether variation between sheep is due to differences in iodine uptake from ingesta to blood or from blood to thyroid glands.

Variability Between Sheep in Iodine Uptake from Potassium Iodide

For sheep 1, 5, and 6, differences in maximal iodine uptake between the first and second doses are appreciable (approximately 15%, 20%, and 15% respectively, in favour of the first dose). For these animals, differences could have been due to individual susceptibility to radiation from previous doses.

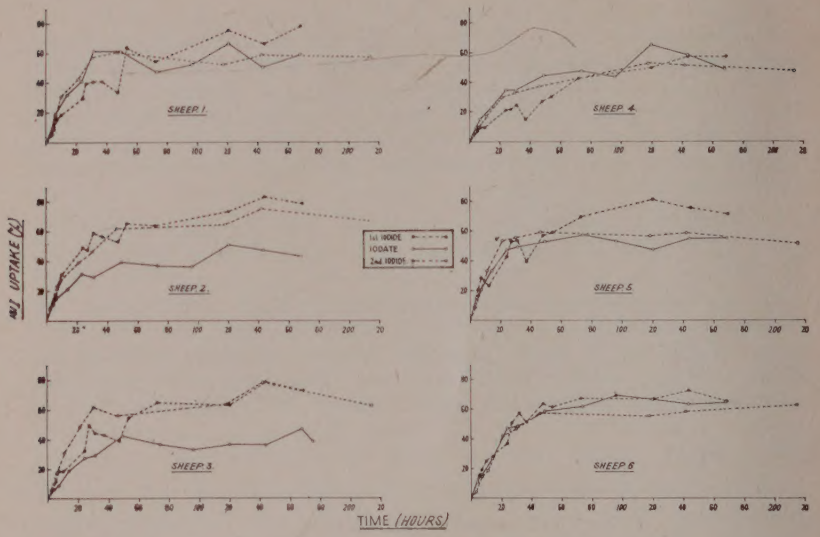

Fig. 1.—Uptake by sheep thyroid glands of iodine from iodate and iodide.

For the remaining three sheep no great change in the pattern of iodide iodine uptake is apparent. This suggests that for these animals factors that might conceivably influence iodine uptake remained reasonably constant during the course of the investigation.

Comparison of Iodine Uptake from Iodate with that from Iodide

For four of the sheep the effect obtained from potassium iodate is comparable with that obtained from either one or both iodide doses. For the remaining animals (Sheep 2 and 3) maximal iodine uptake from potassium iodate was much lower than those from both iodide doses (approximately 45% for iodate doses as compared with 70% for first iodide doses).

These results suggest some degree of individual variation in ability to use iodine from iodate instead of iodide. Nevertheless in all six sheep the amounts of iodate iodine accumulated by the thyroid glands indicate that potassium iodate is a suitable source of iodine for sheep.

ACKNOWLEDGEMENTS

We are indebted to Mr. A. L. Bryant for the graphs shown in Figure 1, and to Mr. B. J. Roddick for technical assistance.

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MILK COMPOSITION STUDIES IN THE HORMONAL INDUCTION OF LACTATION USING IDENTICAL TWIN DAIRY CATTLE

By DAWN R. PERRIN, Ruakura Animal Research Station, Hamilton

(Received for publication, 23 May 1955)

Summary

Changes in milk composition have been studied with identical twin heifers brought into lactation by treatment with progesterone and synthetic oestrogen. In general, the mammary secretions were initially colostral in nature, but, as the milk flow became established, gradually altered to a milk of normal composition as regards fat, protein, lactose, and mineral constituents.

INTRODUCTION

Many investigations have been made on the induction of lactation by hormonal preparations, but few detailed studies are reported of the chemical composition of the resulting secretions as the milk flow becomes established.

The studies by Folley, Scott Watson, and Bottomley[1] with twin goats, and Folley and Malpress[2] with heifers, indicated that the secretion induced by treatment with oestrogens remained colostral in nature for several weeks, and then changed gradually to a milk of normal composition as regards fat, protein, and lactose contents.

The availability of identical twin heifers brought into lactation by progesterone and synthetic oestrogen therapy provided material for a further chemical study of milk composition, both in the initial secretion and as lactation advanced.

EXPERIMENTAL

Seven sets of identical twin heifers were used. These sets were split so that a comparison was obtained between lactation induced by combined progesterone and oestrogen treatment and that of the mate after normal calving. The mammary growth was induced by treatment over 120 days with diethyl stilboestrol and progesterone in the ratio 1,000 to 1. To initiate lactation, increased amounts of diethyl stilboestrol were introduced for 40 days during the final 10 days of which no progesterone was administered. Experimental details of the treatment have been described in an earlier report[3]. Daily injection of diethyl stilboestrol was continued as long as the animal's production increased.

For the animals in which lactation was induced by hormone treatment, milking was commenced the day the animal's co-twin calved, that is, approximately ten days after the cessation of progesterone injections. Milking was attempted twice daily, at the normal night and morning milkings. Chemical analysis was undertaken as soon as a large enough sample was available. For most of the animals, from about the third day onwards, a composite sample was prepared by combining samples taken at night and morning milkings in the proportion of the respective yields. Once the flow became more normal in composition, the analysis was continued on a weekly basis for the remainder of the lactation.

Fat content was determined by the Gerber Test (British Standards Institution, 1936).

Total solids were determined by drying at 98–100°C. (British Standards Institution, 1951), the solids-not-fat being obtained by difference.

Total nitrogen and non-casein nitrogen were determined by methods similar to those described by Rowland[4], the standard semi-micro Kjeldahl procedure used for all nitrogen estimations.

Lactose was determined by a modification of the volumetric copper reduction method of Lane and Eynon[5], the milk being clarified by 10% lead acetate and saturated sodium fluoride solutions. After several months it was found that insufficient time was available to undertake all determinations and for the remainder of the experiment lactose was estimated by difference.

Ash was determined by drying 10g. samples in an oven at 98-100°C. overnight, and then incinerating in a muffle furnace below 550°C. to constant weight.

Chloride content of the ash was obtained by dissolving the milk ash in hot distilled water and titrating against standard N/10 silver nitrate using potassium chromate as indicator.

Calcium and phosphorus were determined by the procedure described previously[6].

RESULTS

As has been reported elsewhere[3], of the seven sets used in these trials, only four had control animals which recorded normal production values. Detailed chemical analyses on the secretions from these pairs are listed in Table 1.

DISCUSSION

From analyses reported in Table 1, it can be seen that the initial secretions from heifers brought into lactation by hormonal induction are similar to colostrum. The change to normal milk depends on the quantity of the secretion, but the colostral phase appears to last longer than it does after normal parturition. Although the total production values do not in general exceed one-half those of the co-twins treated normally, and the persistency of lactation seemed less, the secretion once established appears to be milk of normal quality.

For most of the sets, the fat and protein contents of the milk of the treated animal were higher than in the corresponding samples from the twin in normal lactation. In all cases the values obtained were within the range for normal chemical composition. In mineral constituents, the values obtained for the milk from the injected animals were very close to those obtained for the milk of the twin lactating under normal conditions.

It is difficult to compare the milk composition with similar analyses reported by other workers because the duration and type of treatment have differed considerably. When heifers had implantations or were fed synthetic oestrogens, all animals secreted measurable amounts of fluid within a few weeks from the commencement of the treatment,

TABLE 1. COMPOSITION OF MAMMARY SECRETIONS FROM HEIFERS IN NORMAL AND HORMONE-INDUCED LACTATIONS
(Expressed as percentage by weight.)

Twin No.	Treatment*	Days in Milk	Daily Yield (lb.)	Fat	Protein	Lactose	Ash	Ca	P	Cl
T241	E.P	1**	0.8	1.72	18.24	3.19	1.103	0.222	0.146	0.195
		2**	0.3	2.88	13.94	3.03	1.004	0.194	0.142	0.157
		3	1.2	2.14	11.32	4.84	0.996	0.198	0.154	0.109
		4	5.0	3.90	6.26	4.17	0.870	0.159	0.122	0.085
		6-60 (8)	17.4	4.75	3.83	5.17	0.749	0.127	0.107	0.071
		61-120(9)	15.0	5.30	4.21	4.73	0.733	0.131	0.109	0.070
		121-180(8)	13.2	6.00	4.28	4.53	0.739	0.148	0.107	0.072
		181-240(9)	8.8	6.40	4.84	3.93	0.737	0.133	0.101	0.093
		241-259(2)	5.5	7.65	5.62	2.84	0.782	0.140	0.099	0.117
T242	N.	1**	—	5.20	9.42	3.78	0.930	—	—	0.083
		2**	—	1.60	5.16	4.57	0.830	—	—	—
		3	—	0.55	4.66	4.32	0.804	—	—	0.102
		4	17.0	4.35	4.58	4.25	0.751	—	—	—
		6-60 (8)	26.6	3.75	3.88	4.86	0.733	0.131	0.113	0.066
		61-120(9)	27.2	4.70	3.76	5.15	0.746	0.124	0.101	0.065
		121-180(7)	24.0	5.10	4.35	4.41	0.754	0.137	0.110	0.075
		181-240(9)	21.9	5.10	4.17	4.64	0.748	0.139	0.107	0.075
		241-305(10)	17.4	5.80	4.93	4.03	0.767	0.144	0.103	0.088
T262	E.P	1**	1.4	4.12	8.51	3.76	1.004	—	—	0.199
		2**	0.4	4.49	9.47	2.29	0.960	—	—	0.126
		3	0.6	5.55	6.66	3.84	0.893	—	—	0.186
		5	0.8	3.60	5.83	3.90	0.835	0.121	0.113	0.170
		6-60 (8)	9.4	5.25	3.98	5.13	0.791	0.134	0.100	0.072
		61-120(7)	10.7	5.60	4.07	4.41	0.751	0.124	0.107	0.076
		121-180(9)	8.4	6.45	4.04	4.17	0.750	0.134	0.107	0.090
		181-240(8)	6.1	7.30	4.54	3.16	0.764	0.131	0.098	0.127
		241-262(1)	5.2	6.25	4.58	2.82	0.789	—	—	0.142
T261	N.	1**	—	2.33	4.75	4.39	0.797	0.123	0.094	0.082
		2**	—	1.23	4.48	5.01	0.872	—	—	0.093
		4	13.2	2.67	—	—	0.831	0.145	0.109	0.095
		6-60 (8)	21.4	4.70	3.89	4.85	0.793	0.134	0.110	0.069
		61-120(7)	21.9	5.35	4.03	4.68	0.748	0.134	0.115	0.061
		121-180(9)	19.8	5.70	4.14	4.68	0.752	0.143	0.112	0.064
		181-240(8)	16.4	6.30	4.67	4.09	0.748	0.138	0.106	0.071
		241-282(2)	6.5	6.50	4.83	3.62	0.737	—	—	0.079
T268	E.P.	1**	0.3	0.20	29.03	0.35	0.627	0.048	0.035	0.136
		**	0.2	0.49	22.48	2.80	0.810	—	—	—

		2**	0.2	0.56	15.99	3.17	1.013	—	—	0.162
		**	0.3	0.69	11.48	3.92	1.290	—	—	0.099
		3**	1.8	2.68	7.83	4.19	0.971	—	—	0.109
		**	2.0	3.08	7.27	4.09	0.921	—	—	0.079
		4	9.6	3.25	6.03	4.39	0.862	—	—	0.080
		6-60 (8)	15.0	5.50	3.26	5.07	0.694	0.116	0.102	0.063
		61-120(8)	17.0	4.35	3.40	5.24	0.725	0.131	0.106	0.074
		121-180(8)	15.4	4.90	3.72	4.63	0.713	0.124	0.096	0.081
		181-240(9)	14.3	4.73	3.88	4.71	0.744	0.128	0.102	0.098
		241-305(6)	7.6	6.00	5.06	4.00	0.795	0.109	0.101	0.109
T267	N.	1**	2.5	3.05	13.16	3.67	1.053	—	—	0.138
		2	10.0	2.30	8.01	4.30	0.949	—	—	0.123
		3	13.2	1.47	4.80	4.79	0.849	—	—	0.100
		4	19.24	4.30	4.37	4.47	0.791	—	—	0.091
		6-60 (8)	25.4	3.40	2.94	4.95	0.706	0.114	0.102	0.083
		61-120(8)	30.1	3.95	3.17	4.85	0.720	0.127	0.098	0.083
		121-180(8)	27.8	3.85	3.58	4.62	0.729	0.124	0.095	0.089
		181-240(9)	22.9	4.40	3.80	4.41	0.745	0.136	0.099	0.101
		241-305(9)	17.0	5.30	4.45	3.83	0.743	0.136	0.089	0.092
T276	E.P.	1**	—	3.28	16.72	1.01	1.230	—	—	0.283
		**	—	2.77	15.59	1.59	1.159	—	—	0.233
		2**	—	3.55	12.88	2.82	0.976	—	—	0.202
		3	—	5.20	13.22	3.02	0.920	—	—	0.199
		4	1.7	4.82	9.49	3.25	0.845	—	—	0.158
		5	2.3	3.87	5.26	5.34	0.828	0.118	0.108	0.104
		6-60 (9)	12.0	4.50	3.29	4.70	0.712	0.113	0.105	0.076
		61-120(8)	15.6	5.10	3.39	4.99	0.677	0.128	0.099	0.075
		121-180(8)	13.0	5.07	3.68	4.71	0.660	0.116	0.095	0.078
		181-240(8)	9.2	5.47	3.86	4.22	0.691	0.134	0.094	0.099
		241-280(7)	5.9	6.60	4.65	3.17	0.717	0.133	0.094	0.109
T275	N.	1**	—	3.03	7.65	4.07	1.025	—	—	0.124
		2	—	3.60	5.11	4.18	0.900	—	—	0.120
		3	—	3.24	3.91	5.21	0.782	—	—	0.106
		4	17.5	4.79	3.75	4.27	0.786	—	—	0.096
		5	17.3	3.62	—	—	0.744	—	—	0.093
		6-60 (9)	19.0	4.00	3.11	4.92	0.693	0.116	0.097	0.085
		61-120(8)	19.8	5.30	3.28	4.89	0.683	0.131	0.092	0.080
		121-180(8)	17.2	4.90	3.66	4.52	0.695	0.126	0.095	0.077
		181-240(8)	14.7	5.65	3.92	4.52	0.706	0.126	0.085	0.087
		241-280(7)	7.0	7.10	5.18	3.51	0.805	0.157	0.095	0.107

* N = normal lactation. E.P. = oestrogen- and progesterone-induced lactation.

** Single milking only. All other samples are night/morning samples.

The numbers in brackets indicate the number of samples available to arrive at the average figure.

but the rate of increase of the yield was slow. In the Ruakura experiment, once a reasonable flow became established (in about half the treated animals within 3 days), the yield rapidly approached its maximum, and was then followed by a slow decline as in ordinary lactation.

The early samples from T268 are rather interesting. These initial samples were less viscous and unlike the thick honey-like exudate observed as the first secretion of the remaining heifers. Such serous-like fluids had very high protein contents and very low values for fat, lactose, ash, and minerals, calcium and phosphorus. The casein content was 0.1% to 0.2%. Over the course of several samples, there was a steady decrease in non-casein protein and increase in other constituents until a fluid of a composition more usual for colostrum was obtained. Thereafter the usual changes were observed. It is suggested that, in this particular case, there may have been a discharge of mammary lymph which could act as a diluent of colostrum.

Acknowledgement

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SEEDLING STUDIES IN FESCUE-TUSSOCK GRASSLAND

I. SOME EFFECTS OF SHADING, CULTIVATION, AND FROST

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(Received for publication, 17 May 1955)

Summary

At Molesworth Station, Marlborough, New Zealand, at an altitude of 3,000 ft., experiments are in progress to find out why seedlings generally do not thrive in depleted fescue-tussock grassland. Four species of grasses germinated well in spring and autumn, when counted seeds were sown 1½ in. deep between tussocks of *Festuca novae-zelandiae* in places where an annual *Gnaphalium* was abundant. Neither prior removal of existing plants nor shading during early stages of growth had any beneficial effect in a season of good rainfall. Ninety to one hundred per cent of the seedlings were killed during the first winter by the action of needle ice which lifted plants several inches and severed the roots. Comparisons with other microhabitats and other treatments are being made.

INTRODUCTION

Little is known of the seeding, germination, and early growth of any plants in tussock grassland. Buchanan as long ago as 1880 wrote enthusiastically of the possibilities of indigenous grasses for use in depleted areas, but general experience has been that, whatever the species, few plants establish from broadcast sowing of seeds in such places. It has been pointed out (Saxby, 1950) that the problem is not so much what to sow as to find a satisfactory method of establishment. Dunbar (1950) working with five indigenous and five introduced species found that, although all could germinate and produce seedlings under difficult field conditions, the development of the seedlings was very restricted. Little attempt has been made to analyse the basic causes of failure.

In the course of Botany Division work at Molesworth Station in the upper Awatere Valley, Marlborough, sowing trials were made in depleted *Festuca novae-zelandiae* tussock grassland between the years 1944 and 1952. Seeds of many species of grasses were broadcast or lightly covered in diverse sites. Only *Arrhenatherum elatius* established successfully and few seedlings of other species were seen. Rabbits undoubtedly were responsible for some, but not for all, failures. More detailed studies were planned to discover just what limited germination and establishment. The effects of shading and of disturbance of soil surface were chosen as the first factors to be studied, the methods being adapted in part from those used by Ellison (1949) in Central Utah. Trials were begun in the spring of 1952 and the initial set of sowings and results are described here.

DESCRIPTION OF AREA

The site of the experiments was at Molesworth Station, on the flat top of a low undulating ridge running west to east, south of the Station homestead (Grid Ref. 588306 Sheet 41 Prov. 1-Mile Series, altitude 3,000 ft.). The soil is mapped as Molesworth gravelly sandy loam (Gibbs and Beggs, 1953). The physiognomically dominant plant,

Fig. 1.—Site of experiment. Covered frames show clearly but the uncovered ones scattered between them are scarcely visible.
Photo. Nat. Publicity Studio.

Festuca novae-zelandiae, forms tussocks about two feet tall (Fig. 1). These tussocks occur singly or in small clumps (or "islands") a foot to a yard across, separated by comparatively bare spaces of greater area. There are about forty other species of plants present, of which *Holcus lanatus* and *Agropyron scabrum* are most important within the tussock clumps, and *H. lanatus, Rumex acetosella, Crepis capillaris,* and species of *Epilobium* are generally distributed between the tussocks (Moore, 1954, Table III, Loc. X). Some other species appear to be affected by strictly local differences in habitat so that it is possible to recognize, by characteristic assemblages of plants, several distinct types of inter-tussock space. These same inter-tussock types appear in comparable situations throughout the fescue tussock grassland of this district.

For the initial experiments, sites for 24 plots were chosen between the tussock clumps, and all were within a single inter-tussock type (easily recognized by the great abundance in summer of a small annual *Gnaphalium—G. japonicum* or a closely allied species, Fig. 2). Species in all plots were listed, giving the following order of frequency for those present in half or more of the plots:— *Rumex acetosella, Gnaphalium japonicum* (?), *Poa lindsayi, Holcus lanatus, Geranium sessiliflorum, Agropyron scabrum, Epilobium hectori, Myosotis versicolor.* The soil was tested with a B.D.H. colorimetric pH indicator; in most plots the pH was 6.0, in a few 5.5, and in a damper one where *Gilia squarrosa* was dominant, 5.25. A mechanical analysis showed the soil to be a sandy loam (Gradwell, 1954, Site I), with enough clay, but not quite enough silt, to rank as a silt loam.

A New Zealand Meteorological Office station is situated within a quarter of a mile of the plots and climatological observations have been recorded there since February 1944. Table 1 summarizes rainfall records for the period of the experiment. The last screen frost in 1952 was on 25 November, the first in 1953 on 22 February, and the last

Fig. 2.—Inter-tussock space with strong growth of *Gnaphalium*, not on site of experiments.

Photo. L. B. Moore.

TABLE 1. RAINFALL AT MOLESWORTH, 1952-53

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	12 mths.
Rainfall (inches)	1.73	5.77	3.36	6.42	1.71	2.22	2.17	2.37	2.20	2.14	2.52	1.22	33.83
Rain days	10	17	17	13	6	8	9	8	13	8	8	8	125

in 1953 on 25 October. The season was better than average for plant growth in this area but the site of the plots was much more exposed than that occupied by the weather station. Some daily rainfall and temperature figures at the weather station in July 1953 are quoted by Gradwell (loc. cit. Table II) and his ground temperature records in the same month (Figs. 10 and 11) were taken very near to the plots.

Station horses and cattle had access to the area at times but in fact frequented it little. Rabbits were abundant in some adjacent gullies but there were few signs that they visited the immediate vicinity of the plots.

METHODS

Plots four feet long by two and a half feet wide were protected by frames of 1½-in.-mesh netting standing 6 in. high. Shading was provided by stitching hessian on to the tops of frames. In plots noted as

cultivated, plants were removed and the soil worked to a fine tilth to a depth of six inches. In uncultivated plots existing plants were disturbed as little as possible.

The four grasses used in the trials were *Agropyron intermedium*, *A. scabrum* (local Molesworth seed), *Bromus inermis*, and *B. maritimus*. These are all species that have performed reasonably well in nursery row trials at Molesworth for some years. Well-filled seeds were selected for sowing.

Each plot was divided into four units, each unit to contain a single species. A board into which 50 two-inch nails had been fixed was used as a compound dibble, so that seeds were sown at a depth of about 1½ in. and regularly spaced. Performance of each individual seedling was followed. Ten extra seeds of each species were sown in a similar manner in each plot to be removed at intervals for study of root development. The 24 plots were sown in sets of four: shaded cultivated; shaded uncultivated; unshaded cultivated; unshaded uncultivated. Three sets were sown on 27 October 1952, a fourth on 21 November, and two more in the following autumn on 8 March. Shading was removed in May. Emergence and survival counts were made in November 1952 and in March, May, and November, 1953. Roots and leaves were examined and measured at intervals. Winter observations on frost phenomena very near the plots are available (Gradwell, loc. cit. Site I).

RESULTS

Growth of root systems and foliage was similar in the four species, and measurements of roots and leaves showed no real differences between treatments. Typical root development of the four species 18 weeks from sowing is shown in Fig. 3. These plants were removed in mid-March 1953, from the earlier of the spring-sown plots.

Fig. 4 shows for each observation date the number of seeds sown, seedlings emerged, and seedlings dead. Germination was on the whole good. Both species of *Agropyron* gave better results from the autumn sowing, *A. intermedium* from the same lot of seed as sown in the spring, and *A. scabrum* from newly harvested seed. With the exceptional season even plants from seed sown in March made quite good growth before the winter.

The devastating effect of winter is obvious. In May all plots showed a strong development of needle ice, those which had been cultivated being most strongly affected. Seedlings were still firmly embedded in the soil with foliage browned by frost.

In July, when daily minimum air temperatures of 23° to 26°F. were being recorded at the weather station, the upper inch of top soil appeared to be raised a few inches in many places on the tops of masses of "comb ice". After a surface thaw, soil between 1½ and 3 in. depths remained frozen. By 30 August the ground was completely thawed out and was very damp. Shrinkage of soil had already taken place, and the surface of the soil in the plots appeared partly cut into pillars by comb ice, and partly smoothed out by running water. Some seedlings were still alive but most appeared dead. Seedlings rooted in detached soil crumbs were common (Gradwell, pers. comm.).

Fig. 3.—Seedlings of *Agropyron intermedium, A. scabrum, Bromus inermis,* and *B. maritimus* (from left to right). Above, removed 18 weeks from sowing illustrating typical leaf and root development. Below, heaved out by frost action during the winter, showing how the few remaining roots have been broken across.

Photo. S. N. Beatus.

In November when the surviving plants were counted, the dead seedlings were lying on the broken soil surface. Many of those still alive were attached by one root only. In all dead plants examined the roots had been broken off at a distance of 2 in. or less from the crown. All the secondary roots had been stripped off and the seminal and larger roots were severed (Fig. 3).

For comparison with the sown grasses, some notes were made on species originally present in the plots. *Rumex acetosella* survived the winter well and within the year produced on the cultivated plots a cover equal to that on the uncultivated, mostly from regrowth of deep-lying roots not removed in the limited working of the soil. *Poa lindsayi,* a small and apparently flimsy grass, was more abundant in spring than in the previous autumn; it produces a great number of slender but wide-spreading roots and some whole plants were lifted entirely by frost needles and settled again with the thaw. Many plants of *Holcus lanatus* lasted through the winter, including some that had grown from seed in plots cultivated in spring. *Wahlenbergia albomarginata* which has long deep rhizomes also survived, as did a few small plants of *Festuca novae-zelandiae, Epilobium hectori* (which dies to the ground in winter),

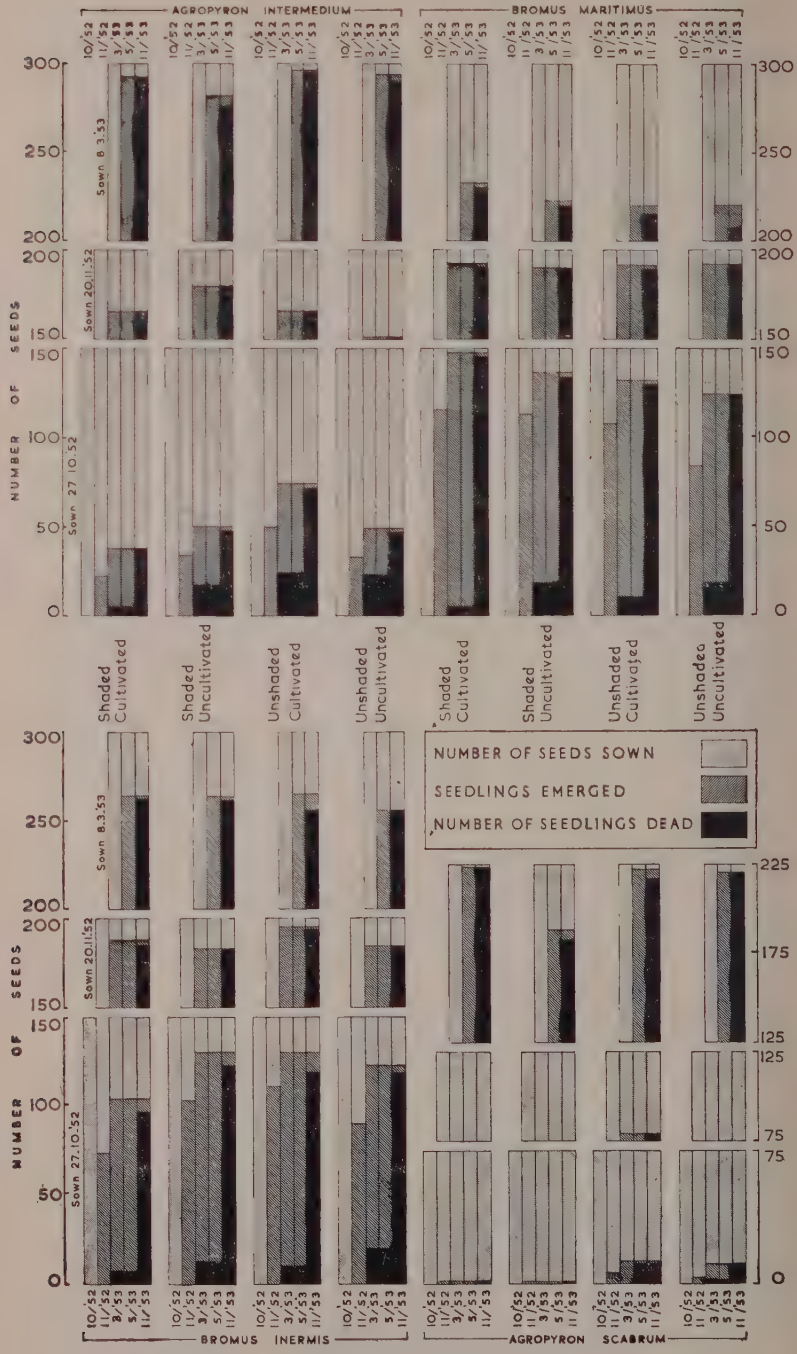

Fig. 4.—Numbers of seeds sown, seedlings emerged, and seedlings dead at each observation date.

Crepis capillaris, Geranium sessiliflorum, and *Poa pratensis.* Nearly all small seedlings of *Verbascum virgatum* were killed but some of *Acaena sanguisorbae* var. *pilosa* survived.

DISCUSSION

Failure of the seedlings to survive the winter in this particular microhabitat can be attributed solely to the heaving action of frost needles. The reduction of competition and the cultivation of the soil had no beneficial effect—in fact there was noticeably more needle ice in cultivated bare soil. Shade in the early growth period was of no advantage in this season of good rainfall. It is suggested that deep or extensive rooting under these conditions is of little value unless it is combined with tensile strength in the roots.

Additional plots have since been laid down in four other microhabitats, and the effect of litter on seedling establishment is also being investigated. There are indications that the type of inter-tussock space chosen for the first experiments has a particularly severe development of frost needles, and that spacing of seedlings has some influence on results.

ACKNOWLEDGEMENTS

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RESISTANCE OF TOBACCO VARIETIES TO BLACK ROOT-ROT

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(Received for publication, 10 May 1955)

Summary

Forty-three tobacco varieties, twenty-two breeding lines, and three *Nicotiana* species were grown in seedling beds infected with *T. basicola*. Infected material was graded into four classes based on visual examination of the roots of thirty plants in each line. Classification of resistance closely agrees with field performance of the material.

INTRODUCTION

Black root-rot, caused by the fungus *Thielaviopsis basicola* (Berk. et Br.) Ferraris[1], is the most serious single factor reducing yield in the New Zealand tobacco crop. In the seedling bed the disease can be controlled by sterilization with steam or methyl bromide[2], but at present there is no treatment that will give economic control of the disease in the field. At the Tobacco Research Station, a breeding programme to combine resistance to this disease with desirable agronomic type is well advanced. During 1951, 1952, and 1954, as part of this breeding programme, the tests reported were carried out to determine rapidly at an early stage of growth the degree of resistance of each breeding line and new variety. Although every variety was not grown each season, several control plots of Harrison's Special 215 were included each year, and all evaluations were made against this standard.

EXPERIMENTAL

Steam-sterilized tobacco seedling beds were inoculated with *T. basicola*[3]. In the 1951 and 1952 seasons, plate cultures of the fungus grown on potato dextrose agar were cut into centimetre squares and these were planted one inch deep in the soil at three-inch intervals. In the 1954 season, a sand and oatmeal culture of *T. basicola* was prepared. This was broadcast uniformly on the soil surface and chipped in thoroughly with a rake.

A 3-8-8 fertilizer was applied at the rate of $\frac{3}{4}$lb. per square yard. Lines under test were seeded in rows six inches apart and grown under normal commercial conditions[4].

When the plants were ready for the field, thirty plants from each line were lifted, and the roots were washed free from soil and examined in water against a white background. Using this technique, infection with *T. basicola* can be readily recognized by the presence of characteristic blackened lesions on infected roots. The presence or absence of *T. basicola* in each line was confirmed under the microscope.

Infection was graded into the following classes:

Nil: No Infection.

Slight: A slight blackening, usually at the tip of secondary roots. Infected region not more than $\frac{1}{2}$ in. in length. No more than two roots infected.

Medium: Several secondary roots showing infection. Blackened areas easily discernible, usually more than 1 in. in length.

Severe: Primary and secondary roots infected. Blackened areas most marked and encircling the root. Secondary and sometimes primary roots reduced to blackened, withered stubs.

Root systems showing typically infected material from each line were mounted and photographed.

Results

Germination in most lines was good. Early growth was slow, but in most lines later growth was much more rapid. Infection seems to

TABLE 1. Infection of Varieties with *T. basicola*

Variety*	Infection per cent. Nil.	Sl.	Med.	Sev.	Variety*	Infection per cent. Nil.	Sl.	Med.	Sev.
					Highly Resistant				
N. debneyii	100	—	—	—	Jadel	100	—	—	—
N. debneyii x *N. tabacum*	100	—	—	—	Little Dutch	100	—	—	—
N. rustica	100	—	—	—	Delcrest 267	100	—	—	—
Xanthi	100	—	—	—	4 Br. lines	—			—
					Resistant				
Delcrest 544	73	27	—	—	T.I. 448A	55	45	—	—
Jamaica Wr.	73	27	—	—	Kentucky 34	43	57	—	—
Virg. Gold 416	37	63	—	—	Haronova	63	35	—	—
Virg. Gold 529	20	80	—	—	Big Jim	63	37	—	—
Virg. Gold 530	20	80	—	—	13 Br. lines	—	—	—	—
Bottom Special	80	20	—	—					
					Moderately Susceptible				
H. Special 523	33	52	15	—	Virg. Gold 539	27	70	3	—
Hick's 545	40	53	—	7	Vamorr 48	17	66	17	—
Hick's 537	43	51	3	3	Vamorr 50	20	77	3	—
Hick's 420	60	30	10	—	D B 101	30	53	17	—
Mortgage Lifter	77	20	3	—	Vesta 11	50	40	10	—
Three Sucker	70	23	7	—	Burley 428	—	70	30	—
Virg. Gold 550	27	70	3	—	4 Br. lines	—	—	—	—
					Susceptible				
H. Special 215	—	18	40	42	D.B. 102	23	37	40	—
H. Special 421	16	36	45	3	T.L. 106	—	23	37	40
H. Special 218	—	26	38	36	Judy's Pride	13	60	27	—
Golden Harvest	10	32	32	26	C. 10/46	10	23	44	23
Golden Cure	—	45	26	29	C. 7/46	7	20	50	23
C	9	18	41	32	Vesta 46	40	40	13	7
Special 402	3	35	12	50	Vesta 47	33	27	40	—
Special 400	13	30	37	20	1 Br. line			—	—
Carol. Bright	—	19	36	45					

*Abbreviations: Wr. = Wrapper. Virg. = Virginia. H. = Harrison's. Carol. = Carolina. Br. = Breeding.

have had little effect on growth. The growth of some of the most heavily infected plants was superior to that of uninfected material in the same line. Infection was uniform over the whole bed, heavily infected material being obtained from both the edges and centre of the seedling beds. There was a marked variation in root development, according to variety. Bottom Special, Delcrest, Xanthi, and several breeding lines, produced a large vigorous, and fibrous root system, while others such as *N. debneyii, N. debneyii* x *N. tabacum*, T.I. 448A, and Virginia Gold, developed only one primary and few secondary roots. There appeared to be no correlation between vigour of roots and degree of infection.

The amount of infection in the material is given in Table 1.

DISCUSSION

Material in the susceptible group is readily infected under field conditions with a resultant decrease in yield. Lines in the moderately susceptible group, given favourable conditions, readily outgrow attacks by the fungus. Varieties in the resistant class are rarely attacked under normal growing conditions. The highly resistant group, which has provided the parental material for the Station breeding programme, has not shown infection under field conditions. (Jadel, a new introduction, has not yet been grown in the field).

Several varieties exhibit greater resistance in the field than the results of these seedling-bed tests indicate. Special 400, Special 402, and Burley 428, for which resistance is claimed overseas, and which have shown good growth when planted in naturally infected areas in the district, fall into the susceptible groups. These varieties are gradually being replaced by more resistant material in tobacco-growing areas overseas.

Harrison's Special 215, which has been the most widely grown variety in New Zealand in recent years, has not shown any greater resistance to attack by the fungus than Harrison's Special 218 in these tests. In the field, Harrison's Special 218, a narrow-leaved variety, is more severely affected by black root-rot than Harrison's Special 215, a broad-leaved variety.

ACKNOWLEDGEMENT

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THE BREEDING SYSTEM OF NEW ZEALAND FESCUE-TUSSOCK *Festuca novae-zelandiae* (Hack.) Cockayne

By H. E. Connor and Audrey B. Cook, Botany Division, Department of Scientific and Industrial Research, Christchurch

(Received for publication, 3 May 1955)

Summary

The breeding system of *Festuca novae-zelandiae*, a perennial grass indigenous to New Zealand, has been determined by comparing the height and culm production of self-pollinated and open-pollinated generations. Inbreeding depression is notable, and indicates that *F. novae-zelandiae* is an outcrossing species, with some slight seed-setting capacity under self-pollination.

Introduction

Fescue- or hard-tussock, *Festuca novae-zelandiae* (Hack.) Cockayne, is an indigenous perennial grass, common throughout the tussock grasslands and associated plant communities on the eastern side of the main divide in South Island, and in the mountains of North Island. Cockayne (1928) lists this species as a physiognomic dominant in the low-tussock grassland. Cockayne and Allan (1934) state that "The name *F. novae-zelandiae* is applied to a very complex group of jordanons and hybrids that have not been satisfactorily analyzed." In the same paper they record possible natural hybridism between *F. novae-zelandiae* and both *F. briquetii* and *F. rubra*. Zotov (1938) also comments on the presence of jordanons in this species. A general description of this species is given by Cheeseman (1925).

This paper reports experiments conducted to determine the breeding system in this species.

Materials and Methods

Plants of *F. novae-zelandiae* raised from seed collected in the wild, as well as wildings transferred to the nursery, have been self-pollinated by the method previously described (Connor and Cook, 1952).

The progeny from self-fertilized seed was planted in a randomized block along with plants raised from open-pollinated seed, used as the controls.

Results

Self-fertility

From a random collection of seed from plants in the wild at Molesworth Station, Marlborough, about 50 plants were grown. Seed from these plants was used to provide the stock for one of these trials. Among the plants raised, three were selfed in 1951–52 and yielded seed for 26 S_1 plants. Another plant of the same collection yielded 12 S_1 plants from seed from self-fertilization in 1952–53. During several years, numerous other attempts to produce seed for an S_1 generation have been unsuccessful, but in the 1954–55 season selfed seed was set on two plants raised from seed from Omarama. On one plant there were 211 filled seeds in a total of 548 florets, and on the other there were 15 filled seeds in 561 florets.

Inbreeding Trial

The inbred Molesworth plants and their open-pollinated control plants were planted out from boxes in the early spring of 1952. Neither generation flowered in 1952–53 season but there was a visible difference in vigour between the two generations, depression being noticeable in the S_1 plants.

During the next season (1953-54) flowering occurred in both lots, 20 S_1 and 25 open-pollinated plants flowering. Measurements were made of the maximum height of the plants, and counts were made of the number of culms per plant. The two generations were noticeably different both in maximum height and in the number of culms per plant. The results from a statistical analysis of these data are given in Table 1.

TABLE 1. Mean Height and Mean Number of Culms of Self-pollinated and Open-pollinated Generations

Season 1953–54	Open-pollinated	Self-pollinated	t
Mean Height cm.	72.3	52.9	7.39**
Mean No. Culms†	1.69	0.93	4.55**

** Significant at 1% level
† Because of the nature of the data, log (1 + culm number) has been used.

Inbreeding depression is particularly noticeable in the self-pollinated progeny in both measurements. No chlorophyll deficient plants were recorded, and there was no apparent segregation. No seed was set in the single attempt to produce seed for an S_2 generation.

In the season 1954–55, 23 S_1 plants and all the open-pollinated plants flowered. The inbreeding depression noticeable from the previous seasons was still prominent, though in both lots there appeared to be more culms per plant than in 1953–54. A further attempt to obtain seed for an S_2 generation was unsuccessful, though S_1 plants set ample seed under open-pollination.

Discussion

The demonstration that *F. novae-zelandiae* is an allogamous species is in agreement with results for other perennial *Festuca* spp. (cf. Beddows, 1931; Nilsson, 1934). This knowledge should help in an analysis of the many forms reported from field and herbarium studies.

Conclusions

F. novae-zelandiae showed little self-fertility. Pronounced differences were detected between the first generation from self-fertilization and the open-pollinated generation. The breeding system of this species is cross-fertilization.

Acknowledgement

Thanks are owing to Dr. R. M. Williams, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for arranging the statistical analysis used in this paper.

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AN AERIAL SURVEY OF ASCOSPORE DISTRIBUTION OF BLIND-SEED DISEASE OF RYEGRASS (*Gloeotinia* (*Phialea*) *temulenta*)

By J. C. NEILL and C. S. ARMSTRONG, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

Summary

Results are given of three years survey, by means of the aeroscope, of the period and range of aerial distribution of ascospores of *Gloeotinia* (*Phialea*) *temulenta*.

Observations are made on the use of the aeroscope for general biological analysis of air.

INTRODUCTION

The air surveys here recorded were designed to obtain information on two questions of primary importance in the study of blind-seed disease of ryegrass (*Gloeotinia* (*Phialea*) *temulenta* (Prill. et Delacr.) Wilson, Noble, et Gray[1]): (1) Is there a generalized distribution in the regional air of the ascospores of *Gloeotinia* responsible for primary infection? (2) Over what period in relation to the flowering period of ryegrass does ascospore discharge occur? A further object was to investigate the use of the aeroscope for biological analysis of air.

THE AEROSCOPE METHOD OF AIR ANALYSIS

Impact methods of trapping air-borne spores on prepared slides are unsuitable for use with *Gloetinia* ascospores because of the difficulty of indentification by direct observation. Only by germination and culture can they be distinguished from the spores of other fungi normally present in the atmosphere. Some approximation of their relative adundance can be obtained by simple petri-dish exposure but results are not satisfactory and moreover yield no quantitative measure of the air load. For the present work a method was adopted based on the use of an apparatus made by the Dominion Laboratory for use in biological analysis of the air in meat works. Subsequently it was found that a similar apparatus had been used with success in the U.S.A. under the name "Aeroscope"[2].

In essence the aeroscope consists of an air pump calibrated to draw a measured volume of air through a tube whose perforated base is immersed in sterile water contained in the bulbous base of an outer tube. An aliquot portion of the water is then spread on nutrient media, incubated, and the resultant colonies recorded.

In adapting the method for use with *Gloetinia* it was necessary to evolve a culture medium which, while encouraging the ascospores to germinate and develop sufficiently for positive identification, would inhibit or restrict the growth of other air-borne organisms. The medium chosen for the present work consisted of normal Dox-Czapek in which the sugar was replaced by yeast extract (marmite). On this, the common air-borne fungi, such as *Cladosporium,* and most bacteria, form small restricted colonies with minimum masking of the *Gloeotinia*. In the field work, 10 cu. ft. of air was bubbled, at approximately 1 cu. ft. per minute,

Fig. 1.—The Aeroscope.

through an initial 7.5 ml. of water, reduced to about 6 ml. during the run; and 0.5 ml. of this spread immediately on each of 6 petri dishes and incubated at 23°C. for 6 days. Initial sterilization of the complete bubbler unit, with both orifices plugged, ensured freedom from contaminants. *Gloeotinia* ascospores germinate in a characteristic pattern, the primary germ tubes growing from each end of the spore outwards for an appreciable distance before developing lateral branches, thus being readily distinguished from the circular colonies of most other fungi. Moreover in 6 days on this medium the fungus produces abundant conidia so that doubtful cases can be identified with certainty under the microscope.

FIELD SURVEYS

On 25 November 1952, samples of air were taken with the aeroscope on the roof of a building 60 ft. high, and at ground level in the central square of Palmerston North city, at least a mile from any ryegrass area. In every case *Gloeotinia* was present in the samples. It would appear therefore that the spores responsible for primary infection of ryegrass with blind-seed disease have, during the period of apothecial discharge, a generalized distribution in the regional atmosphere.

In March 1952, a special plot of 1/8 acre was sown at the Grasslands Division research area with New Zealand certified perennial ryegrass seed heavily infected with blind-seed disease. This was left undisturbed for three years 1952 to 1954, except for periodical grazing with sheep. The aeroscope was set up within this plot and samples of 10 cu. ft. of air taken daily at noon from October to December each year. The results are presented graphically in the lower histogram of Fig. 2 below, the columns representing the mean number of ascospores recorded for three day periods. In 1954 a daily 5 minute search was made for open apothecia of *Gloeotinia* in the plot during the aeroscope run. The numbers recorded are presented, also as a three day mean, in the upper histogram.

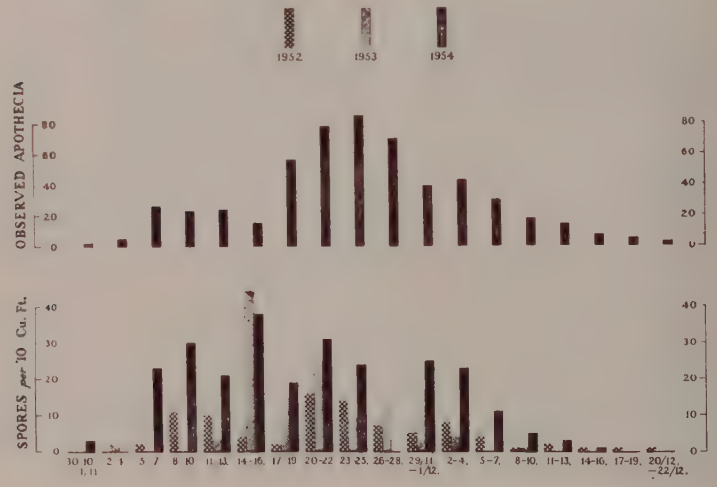

Fig. 2.—*Upper:* Numbers of open apothecia observed in the field. *Lower:* Numbers of ascospores trapped in the Aeroscope.

It is apparent from Fig. 2 that the period of ascospore discharge by the apothecia of *Gloeotinia* in this locality lies between early November and mid December. The peak period, when major infection is likely, covers about one month—from 5 November to 5 December. This coincides closely with the flowering period of perennial ryegrass and, since primary infection takes place almost entirely by ascospores entering open florets, it would seem reasonable to assume that types of ryegrass with flowering periods two or more weeks earlier or later would largely escape the disease. Supporting this assumption is the fact that the later-flowering Italian and hybrid ryegrasses are rarely heavily infected in the field, though equally susceptible under artificial infection in the laboratory. The implications of this to the plant breeder have been fully described by Corkill[3].

NOTES ON THE AEROSCOPE

The aeroscope has, in the present work, furnished a clear definition of the period during which primary infection of ryegrass with blind-seed disease may be expected to occur. It has also demonstrated that there is a generalized distribution of infective spores in the regional air. There

are, however, limitations to its use as a tool in air analyses for general biological problems. Obviously the organism sought must be capable of laboratory culture and, if so, must be readily identifiable therein. Moreover, efficiency of capture varies with the type of infective material. It was found in laboratory tests of enclosed atmospheres containing spores from pure culture that, if three bubbler tubes were placed in series, non-wettable spores such as the conidia of *Cladosporium* and *Penicillium* were caught in the proportions of about 75% in the first tube, 20% in the second, and 5% in the third. On the other hand no *Gloeotinia* ascospores were found in the water of the second tube, presumably because of their wettable mucoid coating.

In field work, results are very much affected by the weather. Rain within the previous twelve hours greatly reduced the catch of *Gloeotinia* ascospores, which dropped to zero if rain fell during the run. To this factor can be attributed the irregular outline of the histogram in Fig. 2, since rain fell during the run, or within the previous twelve hours, on 20 days in 1952, 18 days in 1953, and 25 days in 1954, coincident with the days of lower catch during the peak period. If due allowance is made for this factor, there is a definite time correlation between the number of ascospores caught by the aeroscope and the number of apothecia observed in the plot (Fig. 2).

It is of interest that *Cladosporium*, the commonest and most easily recognized of the other air-borne fungi, was caught in fairly constant numbers throughout the whole series.

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PASTURE ESTABLISHMENT ON PUMICE SOILS

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Summary

In the large-scale development of pumice soils in the central plateau region of the North Island of New Zealand good pastures are being produced, but the establishment stage on much of the area is prolonged owing to lack of thrift and high mortality of the clover seedlings.

On these light soils, highly deficient in both phosphate and nitrogen, the following practices used in combination were found to give the vigorous early clover growth needed in order to improve the rate and efficiency of pasture establishment:

1. The drilling of the seed ¾ in. deep on a well consolidated seed bed, with placement of phosphate fertilizer close to the seed. A roller drill was especially developed for this purpose.

2. Inoculation of the seed. Insufficient effective rhizobia are present in the soil to ensure adequate nodulation of clover seedlings, and inoculation eliminated the early check to clover growth.

Of the methods of inoculation tried, those found successful and suitable for application to mixed seed on a large scale were:

(1) mixing with an inoculum grown on finely divided peat or pumice, and (2) treatment with inoculum suspended in milk at such a rate that, after vigorous mixing, all the free liquid was absorbed by the seed within a few minutes. In order to ensure adequate nodulation, high rates of inoculation were required.

Using the above practices, better and more uniform emergence of seedlings was obtained, with better survival and growth of the clover seedlings, particularly of white and subterranean clover. As a result it was possible to reduce both the rate of seedling and of initial fertilizer application and still obtain better seedling estabilshment and balance of clovers as compared with normal broadcast methods.

Some implications of these results are discussed.

Introduction

In the early years of European settlement in New Zealand little agricultural development took place on the large area of land deeply covered by Taupo and Kaharoa volcanic ash showers, notwithstanding the favourable climate and topography (N.Z. Soil Bureau, 1954). Those who attempted to establish pastures on this land found that only the less productive grasses persisted and that young stock suffered from a wasting disease which they called bush sickness. In view of that experience it was generally concluded that the land was more suitable for forestry than for farming, but two discoveries made about 25 years ago changed the outlook markedly. Following the work of Filmer and Underwood (1934) it was shown that bush sickness was due to cobalt deficiency and could be prevented by the application to the land of only a few ounces of cobalt salts per acre. At the same time Smallfield (1933) demonstrated at Ngakuru and Galatea that good permanent pasture could be established directly on virgin pumice soils by the use of persistent strains of perennial ryegrass and white clover, and heavy applications of superphosphate.

The agricultural development of pumice land was thereupon commenced on a large scale by the Departments of Lands and Survey, and of Maori Affairs, and it is still proceeding at an increasing rate. The native vegetation is crushed with rollers and burned, and the land is then cultivated in the normal way to prepare a seed bed (Fig. 1). Seed is broadcast and rolled in, and cobaltized superphosphate is broadcast at the rate of 3 cwt. per acre. Two similar applications of superphosphate are made at intervals of about 6 months and thereafter the pasture receives an annual dressing. The ordinary weeds of agricultural land are at first uncommon and re-growth of fern and scrub is effectively controlled by heavy stocking with wethers and steers.

A survey of pastures established on virgin pumice soil was commenced by the senior author in 1949. It was found that generally the pastures grew poorly at first, passed through a stage of red-clover dominance, and finally, at least two years after sowing, developed into pastures comprising mainly perennial ryegrass, cocksfoot, and white clover, with small amounts of red clover.

Almost everywhere a large proportion of the clover seedlings, especially those of white clover and subterranean clover, ceased to grow with normal vigour at about the time the first trifoliate leaf appeared. The subsequent development of the pasture depended on the growth of the few clover seedlings which escaped or survived this check, and on the natural increase of these plants by re-seeding. It appeared that if the survival rate and speed of growth of clover seedlings, especially white clover, could be improved, pastures with a good balance of grass and clover might be obtained more quickly and with more economical use of seed.

Fig. 1.—Surface of ground just after being sown by the normal broadcast method. Three very different seedbed surfaces presented—rolled surface, marks left by seed trailer, and crawler tractor marks.

The phase of extreme red-clover dominance in the normal development of new pastures on the pumice has two deleterious effects. Bloat becomes a serious problem, and the development of the surviving plants of the other sown species is prejudiced through being overshadowed by red clover. The effect is intensified, and the period of red-clover dominance is prolonged, if the pastures are cut for hay without the normal return of dung and urine by the grazing animal.

An investigation into the causes of clover seedling unthriftiness was therefore undertaken, and experiments in pasture establishment were commenced in 1950. Studies were pursued concurrently along three lines: inoculation, plant nutrition, and methods and rates of sowing. In the early exploratory stages, pumice soil was taken to Palmerston North for pot studies, and simple experiments were laid down in the field. When the main issues had been defined, more elaborate field experiments were undertaken.

Climate, Soil, and Vegetation

The pumice land extends from Lake Taupo to the Bay of Plenty and lies from 600 to 2,000 ft. above sea level.

Meteorological records from Rotorua indicate the main features of the climate. Annual rainfall is about 54 in. and is evenly distributed through the year. The average daily maximum temperature in January is 75°F. and in July 55°F. The average daily minimum temperatures for those months are 53°F. and 37°F. respectively.

The soils are derived mainly from rhyolitic ash deposited during volcanic eruptions within the past 2,000 years (Baumgart, 1949). Being of such recent origin the soils have undergone little weathering. They vary in texture from fine sands to coarse stony soils, and are all light and free draining. All except those derived from coarser ash and water-sorted material found in the depressions have a moderately good water-holding capacity. Most of the area is covered with tussock grasses, fern, and scrub, but the climax vegetation would appear in general to be rain forest.

Preliminary Work

The surface of pumice soil dries very quickly but never coheres to form a crust. In glasshouse experiments it was found that even the smallest seed used, white clover, could be sown in pumice at a depth of $\frac{1}{2}$ to 1 in. with marked advantage under dry conditions. It was also found that clover seedlings responded more to superphosphate drilled with the seed than to the same fertilizer broadcast on the soil surface (Fig. 2). It was concluded that drilling the seed with fertilizer could be advantageous, but it was obvious that the use of a coulter drill would be impracticable because of the abundance of sticks and pumice boulders and the unevenness of the surface. A roller drill was therefore constructed in collaboration with Mr. Cross of Massey Agricultural College.

The implement was designed to sow seed at a depth of about $\frac{3}{4}$ in. in compacted drills 3 in. apart, at the same time giving even distribution of seed and placement of most of the fertilizer close to the seed. It consisted of a ring roller with seed and fertilizer boxes mounted behind (Fig. 3). The roller wheels were cast using a normal Cambridge wheel base pattern (24 in. diameter), but with the solid rims built up to give

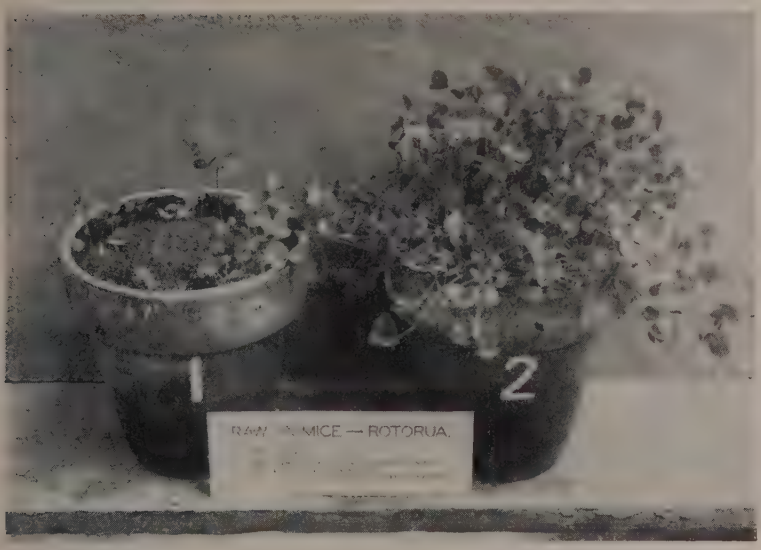

Fig. 2.—Effects of seed and fertilizer placement seen in the early glasshouse pot trials.

Fig. 3.—Prototype roller-drill with special wheels for definite tracking, and seed and fertilizer boxes mounted to distribute directly into the roller marks.

Fig. 4.—Seed bed left by roller drill. Normally, harrows or a Cambridge roller follow immediately behind, covering the seed and fertilizer before excess drying out of the soil surface.

a triangular profile with the apex of the triangle 2 in. above the 3-in. wide rim. Such a shape met the requirements of optimum depth of sowing and of lateral compaction of the pumice soil needed to hold the shaped grooves until the seed and fertilizer had fallen into position. The final operation is covering with a trailed chain, brush harrow or, preferably, Cambridge roller to give additional surface compaction (Fig. 4). Another advantage of the machine is the better crushing of pumice boulders, both by the sharp edges of the rings and their greater weight as compared with those of Cambridge roller.

The roller drill was first used on pumice land at Reporoa in 1952. The seedling stand where a seed mixture was drilled at 26 lb. per acre appeared as good as that where the usual 45 lb. of seed was sown in the normal way, and it was superior in evenness of distribution.

In addition to these early tests on seeding methods and rates, trials of nitrogen, phosphate, micro-nutrients, and of small additions of soil from a local paddock of well-established white clover, were carried out. From these trials it was concluded that (a) inoculation of the clover seed with effective strains of *Rhizobium* would probably give better clover establishment, and (b) no mineral element other than phosphate was limiting early growth. The former conclusion had been reached not only from the small trials with soil from the local clover paddock but also from the fact that in trials using uninoculated seed, an addition of nitrogen (urea) at seeding time had given a transitory, although definite, response in the clovers, thus indicating that the rhizobia present in the virgin soil were either ineffective, or were present in insufficient numbers for satisfactory inoculation of the sown clovers.

These preliminary results led to a more detailed field experimental programme, first at Wairakei, and later at Tauhara. Details of the results from these trials are given in the following two sections.

Section 1: Effect of Inoculation

On examining seedlings of subterranean, white, and red clover during the early check to growth in the field it was found that the checked plants had no nodules on their roots while the few normal seedlings were nodulated. In preliminary work (Johns, unpublished) it had been shown that some rhizobia effective or partially effective on clovers were present in these virgin pumice soils. The rhizobia were possibly those associated with the occasional plants of several clover species which had been naturally introduced into the native scrub growth. However, the lack of nodules on a high proportion of the seedlings of the newly sown clovers suggested that the numbers of rhizobia present in the soil were insufficient to ensure nodulation of all seedlings, and that this failure to nodulate was the cause of the check to growth of the seedlings.

This was confirmed in a plot experiment laid down in October 1952 in an area fenced off in a new development block near Wairakei. In this trial, vigorous and continued uniform growth of the clover seedlings was obtained in the inoculated plots, while in the uninoculated plots the seedling growth was variable and in most cases stunted. These differences were more marked with subterranean and white clovers than with red clover. A strain of *Rhizobium* supplied by Dr. W. D. Reid of the Plant Diseases Division, which was used for inoculating white clover in the trial, was later found to be effective on red and subterranean clovers also. This strain has been used in the further work on clover inoculation described in this paper.

In the field plot experiment referred to above, it was shown conclusively that inoculation was effective in avoiding the early check to growth of the clover seedlings. It was then considered necessary to examine methods of inoculation that would be suitable for use on a large scale where the grass and clover seed is mixed in bulk beforehand, and to estimate the degree of benefit likely to be obtained from inoculation under the practical conditions of land development.

Several methods of inoculation of legume seed have been used (Fred, Baldwin, and McCoy 1932; Lochhead 1932), but many of them are not readily applicable on a large scale to mixed grass and clover seed. The method of using soil from an area growing good clover is not suitable owing to the large amount of soil—4 cwt. or more per acre—that is required in order to get good nodulation.

A method widely used for legume inoculation is that of thoroughly damping the legume seed with a suspension of a pure culture of a suitable strain of *Rhizobium*, drying in the shade, and sowing without delay. Usually the bacteria are grown on nutrient agar, and suspended in skim milk, to which sugar (Lochhead 1932), calcium phosphate (Thornton and Gangulee 1926), or calcium carbonate may be added. This method is not suitable for use with mixed grass and clover seed on a large scale, but as it is a well-tried method, and is the one used for lucerne in this country, it was used in the experiments for comparison with other methods. A possible modification of this method, applicable to mixed grass and clover seed, is to add just sufficient milk inoculum (about 3% by weight) to ensure that after the seed has been vigorously mixed for a few minutes (e.g. in a concrete mixer) all the free liquid is absorbed (largely by the grass seed), and the seed again

flows freely. The seed should then be sown without delay, to avoid both a loss of viable rhizobia and a decrease in germination capacity of the seed.

Instead of growing the rhizobia on nutrient agar, they may be grown on sterile finely ground peat, soil, or similar material. This method has been much used in the United States, Sweden, Germany, and elsewhere, and it is becoming more widely used owing to the ease with which the inoculum may be stored (Hedlin and Newton 1948; Newbould 1951; van Schreven, Otzen, and Lindenbergh 1954; Gunning and Jordan 1954). The method of seed inoculation using these cultures is usually a wet method similar to that with agar cultures. The addition of the powder direct to the seed without wetting has been advised against in general (Leonard 1938), though it has been suggested from time to time because of its convenience. It is the method at present used in Australia with commercial cultures, and has apparently recently been found effective in Canada (Gunning and Jordan 1954). This method of dry mixing was tested here for applicability to mixed grass and clover seed.

Experimental

Three main experiments were carried out to test the efficacy of various methods of inoculation:

(1) A trial with a number of small plots, put down on pumice subsoil using white and subterranean clovers sown with ryegrass in October 1953.

(2) A larger scale trial using a normal pasture mixture sown on cultivated pumice in March 1954.

(3) A small-scale trial on subsoil, similar to (1) carried out at the same time as (2).

Experiment 1

Various methods of inoculation were tried on seed sown in small plots in a fenced-off area near Wairakei. The area was part of the Orouanui development block of the Lands and Survey Department, on which scrub had been crushed and burnt, and the soil giant-disced and worked down. In order to get better uniformity of soil conditions and also to reduce the chances of soil-borne rhizobia complicating the results, the disturbed upper soil layer was removed and the plots sown on subsoil, which was extremely deficient in phosphate, nitrogen, and clover bacteria. For marking out the plots a board 2½ ft. long having triangular wooden strips 3 in. apart fastened lengthwise on the under side was used. This board, when pressed into the loosened soil, formed grooves 3 in. apart and about 1 in. deep (Fig. 5). Serpentine superphosphate equivalent to about 2 cwt. per acre was spread along the rows, 3 rows each of white and subterranean clover (both mixed with perennial ryegrass at 20 lb. per acre) were sown in each plot, and the rows were covered in and firmed with a rake. Care was taken to prevent contamination between plots, but as they were not widely spaced some cross contamination was inevitable in time.

The inoculation treatments were as follows:

A. Inoculum suspended in milk, and applied to clover seed only (1 ml. to 24 g. of seed) and the seed dried in the shade and sown within a few hours.

Fig. 5.—Small experimental plot on subsoil pumice being prepared by use of board shaped to simulate roller-drill action.

(1) Level of bacteria given by a half-grown culture on agar in an 8 oz. bottle suspended in milk and diluted to 250 ml.

(2) Inoculum as in (1) but with blood and bone at 1½ cwt. per acre added along the drill rows.

(3) Level of inoculum as in (1) but with the addition of 10 g. sucrose, 1 g. calcium phosphate, and 0.5 g. calcium carbonate per 100 ml. of milk.

(4) Level of bacteria 50 times greater than in (1)—inoculum was very viscous.

B. Inoculum suspended in milk, applied to mixed grass and clover seed at 3% by weight, seed mixed vigorously until all free liquid was absorbed, and the seed sown within a few hours.

(5) Inoculum as in (1).

(6) Inoculum as in (3).

(7) Inoculum as in (4).

(8) Inoculum as in (4) applied to grass seed only, with uninoculated clover seed.

C. Inoculum in powdered peat (U.S.A. origin).

(9) Peat inoculum at high rate (approx. 200 mg. to 12 g. seed), dampened and added as slurry to the clover seed alone, and this then dried and sown without delay.

(10) Same rate as (9) of peat inoculum dampened and added to mixed grass and clover seed.

(11) Peat inoculum at same rate as (9) mixed dry with seed and sown without delay.

(12) As (11) but with inoculum at about 10 times the rate.

D. Inoculum in powdered peat, prepared shortly beforehand at the laboratory.

(13) The same amount of peat inoculum as in (11), mixed dry with seed.

(14) The same amount of peat inoculum as in (12), mixed dry with seed.

E. Inoculum in powdered peat (Australian origin).

(15) The same amount of peat inoculum as in (11), mixed dry with seed.

(16) The same amount of peat inoculum as in (12), mixed dry with seed.

(17) Control, not inoculated.

Duplicate plots of each of these treatments (with 6 uninoculated control plots) were sown on 30 October 1953. On 10 December (41 days) and 11 January 1954 (72 days from sowing), counts of vigorous plants and of total plants were made. The vigorous plants were larger, green, and actively growing, as compared with the non-vigorous plants, which were stunted, yellowish, and with their outer leaves dying back; most plants could be readily classified into one or other of these categories. The results of the counts are given in Table 1, the vigorous plants at each count being expressed as a percentage of the total plants in the first count. The figures given are the true mean percentages, the standard errors being obtained by decoding the standard errors of the transformed means.

In many cases the number of vigorous plants had increased somewhat by the second count, but the total number of plants was in most cases lower, particularly in those plots where the number of vigorous plants in the first count was low. The first count gives more clearly the differences between treatments.

In the white clover treatment (1) there was very bad agreement between the duplicate plots, one plot giving an abnormally high percentage of vigorous plants—presumably due to contamination. A statistical test of the error distribution showed that the treatment should be excluded from the analysis ($p = 0.001$).

TABLE 1. VIGOROUS PLANTS AS PERCENTAGE OF TOTAL PLANTS PRESENT AT FIRST COUNT

Treatment	Count on 9/12/53		Count on 11/1/54	
	White Clover	Sub. Clover	White Clover	Sub. Clover
1. Milk, clover only, low rate		61.5 ± 8.5		71.4 ± 11.4
2. Milk, clover only, low, blood and bone	12.1 ± 2.3	41.0 ± 8.9	13.2 ± 7.5	66.9 ± 11.8
3. Milk, clover only, low, additions	26.2 ± 3.2	90.9 ± 4.8	48.5 ± 10.8	83.1 ± 9.4
4. Milk, clover only, high	28.3 ± 3.3	94.1 ± 4.3	58.2 ± 11.1	95.3 ± 3.9
5. Milk, mixed seed, low	31.8 ± 3.4	16.0 ± 6.6	76.2 ± 9.5	56.0 ± 12.5
6. Milk, mixed seed, low, additions	48.2 ± 3.6	43.9 ± 9.0	75.6 ± 9.4	78.9 ± 10.2
7. Milk, mixed seed, high	66.8 ± 3.4	56.4 ± 9.0	36.6 ± 7.7	88.8 ± 6.0
8. Milk, grass only, high	46.3 ± 3.6	47.3 ± 9.0	31.1 ± 8.8	93.3 ± 6.0
9. Peat (U.S.A.) clover only, wet	22.3 ± 3.0	87.6 ± 5.9	63.8 ± 10.8	85.0 ± 9.0
10. Peat (U.S.A.) mixed seed, wet	17.8 ± 2.8	81.0 ± 6.9	58.5 ± 11.1	91.5 ± 5.2
11. Peat (U.S.A.) dry	27.0 ± 3.2	58.9 ± 8.9	81.6 ± 8.7	87.6 ± 8.3
12. Peat (U.S.A.) dry, high rate	46.0 ± 3.6	93.2 ± 4.5	86.4 ± 7.5	89.2 ± 7.8
13. Peat (N.Z.) dry	94.7 ± 1.6	94.8 ± 3.0	89.2 ± 6.3	87.6 ± 8.2
14. Peat (N.Z.) dry, high rate	97.1 ± 1.2	96.0 ± 2.6	96.7 ± 2.9	98.7 ± 2.2
15. Peat (Aust.) dry	5.9 ± 1.7	16.8 ± 6.6	24.8 ± 9.3	40.0 ± 12.3
16. Peat (Aust.) dry, high rate	11.9 ± 2.3	18.1 ± 6.9	22.8 ± 9.4	40.1 ± 12.4
17. Control	0.8 ± 0.4	0.7 ± 1.0	5.4 ± 4.2	4.6 ± 4.1

By an analysis of variance on the transformed percentages, taking the first count only, the following points were shown: All inoculation treatments were significantly better than the control. The addition of blood and bone in the drill rows gave no advantage. Increasing the level of bacteria (treatment 4) or supplementing the milk transfer medium with nutrient (treatment 3) increased significantly the number of vigorous plants. Milk inoculation of the mixed grass and clover seed proved satisfactory; with white clover it was significantly better than inoculation of the clover seed alone, with subterranean clover significantly worse. When only the grass seed was inoculated, there was satisfactory nodulation of uninoculated clover seed sown in the same drills. The peat inoculation method at the high rates used was at least as good as the milk method, provided the inoculum was good, and dry mixing of the peat with the seed was entirely satisfactory. The locally prepared peat inoculum was particularly good.

Counts of the numbers of rhizobia added in the various methods were not made, but counts of bacteria in the peat inocula were made

Fig. 6.—Response to inoculation of clovers in a preliminary small-plot trial on subsoil pumice at Wairakei: inoculated on right, uninoculated on left.

later by a roll tube dilution method, and showed that the local peat preparation had at least 5 to 10 times as many rhizobia as the other two; the latter being difficult to count owing to the number of other organisms present.

Experiment 2

Various methods of inoculation having proved successful in small plots, a series of large plot trials was laid down in the Tauhara development block of the Lands and Survey Department on 17 and 18 March 1954, those concerning inoculation being experiments 2A and 2B below.

The Tauhara area was chosen by the Lands Department as being typical of the areas on which pasture establishment was most difficult. The area used was in a flat basin having a fairly coarse soil with a compacted subsoil.

The pasture mixture was that used by the Lands Department, and consisted of ryegrass (20 lb.), cocksfoot (6 lb.), and crested dogstail (3 lb.), with red clover (3 lb.), white clover (3 lb.), and subterranean clover (2 lb.), total 37 lb. per acre.

Serpentine superphosphate at 3 cwt. per acre was drilled with the seed in all plots.

The plots were one drill-width (7 ft.) wide, and 80 yards long in experiment 2A, 48 yards long in 2B.

Experiment 2A was a simple comparison with 10 replications of no inoculation versus inoculation at a high rate using combined milk inoculation of the mixed seed and the addition of inoculum grown on pumice.

In Experiment 2B, different rates and methods of inoculation were compared in a random block arrangement with 8 replications. The treatments were:

1. Control, uninoculated.
2. Milk inoculation of mixed grass and clover seed.
3. Milk inoculation of clover seed only, which was then dried in shade and mixed with grass seed.
4. Inoculum grown on finely divided peat, mixed with seed without added liquid at 8 g. per acre.
5. Inoculum as (4) but at 32 g. per acre.
6. Inoculum as (4) but at 128 g. per acre.
7. Inoculum as (4) but at 512 g. per acre.
8. Inoculum as (4) but at 2048 g. per acre.
9. Inoculum grown on finely sieved pumice, mixed with seed without added liquid at 64 g. per acre.
10. Inoculum as (9) but at 512 g. per acre.

The different treatments were sown in the above order, and after each set of treatments was completed, the roller drill was thoroughly cleaned out, and those parts exposed to the seed were flushed with alcohol and allowed to dry before the next treatment was applied.

The plots in Experiment 2A, but not 2B, were rolled with a Cambridge roller after sowing, the control plots being rolled first. The weather was rather dry and windy for some days after the sowing, with some blowing of the surface soil. That this probably led to contamination between plots was evidenced by the fact that the control plots had more nodulated plants than in a similar sowing outside the experimental area, and this should be borne in mind when considering the results.

At the same time as the plots were sown, estimations were made from samples kept at the laboratory, of approximate numbers of rhizobia added in the various treatments, and these are given in Table 2 below.

The plots were examined on 29 and 30 April 1954, emergence counts made, and samples taken for initial nodulation counts. The seedlings were more forward in Experiment 2A, apparently owing to the final rolling, and this advantage was maintained throughout the winter. Much damage by birds to the cotyledons was observed, particularly in red and subterranean clover, few seedlings of these two species having both cotyledons intact.

Emergence figures per square foot over all plots were: white clover 22.6, red clover 3.2, sub. clover 0.7, which is about 38%, 17%, and 20% of the viable seed sown. These low figures were in part due to bird damage. Results obtained with the three species showed no marked differences, and, since the precision in the counts was much greater with white clover owing to the greater numbers, only the figures obtained for white clover are given below.

Further samples were taken for nodulation counts on 14 May and 30 June, and the percentage of nodulated white clover plants in the various treatments are given in Table 2. In the first two counts

all nodulated plants were included, but in the final count only those plants with effective nodules (reddish coloured) were included. At this final count the percentage of seedlings with ineffective nodules (without any reddish colour) varied from 8.4% in the controls to 1.9% in the pumice inoculum at a high rate, and showed an inverse trend to the percentage of effectively nodulated plants.

TABLE 2. PERCENTAGE OF WHITE CLOVER PLANTS NODULATED AT THREE HARVEST DATES (All nodulated plants included in first two harvests, but only those with definitely pink nodules in the third harvest.)

Treatment	Approx. No. of Bacteria per Sq. Ft.	Harvest 28/4/54 (6 weeks)	Harvest 14/5/54 (8 weeks)	Harvest 30/6/54 (15 weeks)
2A				
Control	—	7.2 ± 1.4	21.7 ± 1.51	33.6 ± 3.2
Inoculated	1×10^8	92.9 ± 1.4	97.4 ± 0.53	98.7 ± 3.2
2B				
Control	—	9.2 ± 2.53	26.8 ± 5.3	39.0 ± 3.4
Milk, mixed seed	2×10^6	60.3 ± 3.92	83.4 ± 5.2	89.0 „
Milk, clover only	9×10^5	27.4 ± 3.27	58.0 ± 6.9	68.5 „
Peat 8 g.	2×10^5	19.8 ± 2.74	53.8 ± 7.1	64.5 „
Peat 32 g.	7×10^5	29.9 ± 3.69	67.2 ± 6.6	75.0 „
Peat 128 g.	3×10^6	55.6 ± 4.02	80.8 ± 5.6	85.0 „
Peat 512 g.	1×10^7	51.9 ± 4.00	89.2 ± 4.4	91.5 „
Peat 2048 g.	4×10^7	76.5 ± 3.25	92.6 ± 3.7	95.0 „
Pumice 64 g.	1×10^7	47.0 ± 3.90	78.0 ± 5.9	87.0 „
Pumice 512 g.	8×10^7	62.4 ± 3.78	93.2 ± 3.5	93.0 „

From Table 2 it is seen that the different treatments have resulted in different proportions of nodulated plants, and that the proportions of nodulated plants have increased with time. There is a definite relationship between the number of bacteria added and the percentage of plants nodulated. This is clearly shown in Fig. 7, where the percentage of nodulated plants at 8 weeks from sowing is plotted against the logarithm of the number of bacteria added per square foot. The five rates of application of peat inoculum lie on a fairly smooth curve, with the milk inoculum to clover seed alone lying below this curve, and the milk inoculum to mixed seed, slightly above. The higher rates of peat inoculum, pumice inoculum, and the milk inoculum to mixed seed could all be considered satisfactory, but high rates of application are required to obtain nodulation of 80% of the plants at 8 weeks from sowing. Considering that in this experiment more care was taken with the inoculation than might be expected from contractors or farmers, 80% does not seem too high an objective.

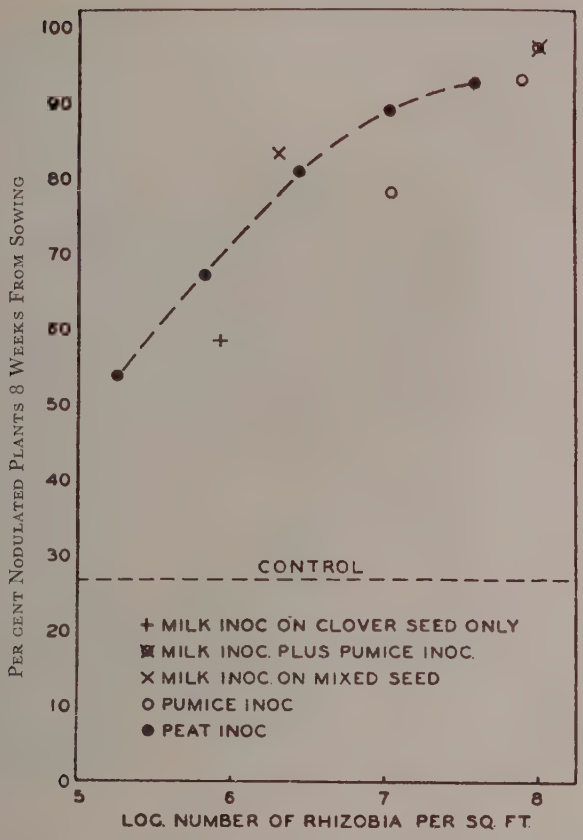

Fig. 7.—Percentages of nodulated plants at 8 weeks from sowing plotted against logarithms of numbers of rhizobia per square foot added in the inoculations.

By 30 June, some seedlings had died, more in those treatments where the percentage of nodulated plants was smaller, but even though marked quadrats had been used for the determination of survival percentages, the variability between plots was too great to allow for adjustments to be made to the percentages of nodulated plants. This effect, and the likely cross-contamination between plots referred to above, would both tend to raise the percentage of nodulated plants in the control and the low-inoculation plots, and thus decrease the differences between high and low treatments; but the extent of these effects, though probably considerable, cannot be assessed.

Plant weights (top plus root) were determined for the effectively nodulated (pink nodules), the ineffectively nodulated or very recently nodulated (white nodules), and the non-nodulated plants, at the harvests on 14 May and 30 June and the mean weights of the effectively nodulated and the non-nodulated plants in these two harvests are shown in Table 3.

TABLE 3. MEAN WEIGHTS (MG.) OF EFFECTIVELY NODULATED AND NON-NODULATED WHITE CLOVER PLANTS AT TWO HARVEST DATES

Treatment	Harvest 14/5/54		Harvest 30/6/54	
	Effectively Nodulated Plants	Non-nodulated Plants	Effectively Nodulated Plants	Non-nodulated Plants
2A				
Control	5.60 ± 0.34	4.72 ± 0.20	8.5 ± 0.77	4.3 ± 0.77
Inoculated	6.34 ± 0.36	2.99 ± 0.21	16.4 ± 0.77	
2B				
Control	4.12 ± 0.50	2.88 ± 0.20	7.10 ± 0.52	3.68 ± 0.32
Milk, mixed Seed	4.30 „	2.82 „	11.70 „	3.80 ± 0.35
Milk, clover only	3.38 „	2.78 „	9.05 „	3.36 ± 0.32
Peat 8 g.	4.16 „	3.30 „	8.85 „	4.42 ± 0.32
Peat 32 g.	4.88 „	3.12 „	9.10 „	3.86 ± 0.32
Peat 128 g.	4.02 „	2.80 „	8.85 „	3.92 ± 0.32
Peat 512 g.	4.38 „	2.32 „	10.45 „	2.86 ± 0.37
Peat 2048 g.	4.16 „	2.10 „	10.35 „	2.80 ± 0.35
Pumice 64 g.	4.03 „	2.70 „	9.45 „	3.12 ± 0.32
Pumice 512 g.	4.06 „	1.80 „	11.00 „	3.02 ± 0.32
Sig. diff. at 5%		0.57	1.45	

In the harvest on 14 May there was no significant difference in the mean weights of the nodulated plants in any treatment in either section of the experiment taken separately. With the non-nodulated plants, however, a reverse trend was shown with increasing rates of inoculation. Thus in the five peat treatments there was a highly significant downward regression of plant weight with increasing rates of peat inoculation ($F = 11.73$ for 1,30 df.). The same effect was evident in the weights of ineffectively nodulated plants, which have not been included in the table.

A possible explanation of this would be that, as the rate of inoculation increased, the greater number of successfully inoculated plants resulted in greater competition for soil nitrogen and phosphorus, against the uninoculated plants.

The harvest on 30 June showed significant differences between the mean weights of the nodulated plants. Thus there was a highly significant upward trend in mean plant weight with increasing rate of peat inoculation ($F = 15.40$ for 1,70 df), and the control was significantly lower than any other treatment. This latter effect could be due to the strain of *Rhizobium* used for inoculation, being more effective on white clover than those strains already present in the soil, but it could also be due to increasing rates of inoculation having caused earlier and more extensive

Fig. 8.—Inoculated and non-inoculated plots in Experiment 2A at Tauhara, on 16th November 1954, showing contrast in growth of clover.

nodulation on the plants, thus increasing the growth of individual plants. In any case it is seen that increased rate of inoculation not only increased the proportion of plants which became nodulated, but also increased the mean weight of these nodulated plants.

The growth of the non-nodulated plants over nearly seven weeks of early winter was very small.

Figure 8 shows the contrast in growth of clover in inoculated and non-inoculated plots of Experiment 2A, photographed on 16 November 1954, after a rather dry spring. In Fig. 9 the extent of growth and nodulation is shown in red clover plants inoculated and nodulated, chance nodulated, and not nodulated, harvested on 7 October 1954.

Experiment 3

Following the laying down of Experiment 2, a series of small plots were sown in the Wairakei experimental area on subsoil, using the same sowing technique as in Experiment 1. This experiment was put down to check methods of inoculation in an autumn sowing, and to check

Fig. 9.—Red clover plants harvested on 7th October 1954, showing effect of inoculation on the distribution of nodules and on the size of plants. On left, inoculated plant from the March sowing, showing well formed nodules grouped mainly just below crown of plant. Centre and right, plants from normal sowing just outside experimental area. The centre plant has by chance become nodulated by rhizobia in the soil, and shows effective nodules grouped on one portion of the root system only. The plant on right was typical in size of those non-nodulated plants that still survived.

whether results from small plots were comparable with those obtained from the larger plot sowings in Experiment 2. At the same time opportunity was taken to determine whether satisfactory nodulation could be obtained with seed inoculated a few days before sowing. The treatments were as follows:

1. Inoculum in milk applied to clover seed alone.
2. Inoculum in milk applied to mixed grass and clover seed.
3. Inoculum grown in peat added dry to mixed seed.
4. Inoculum grown in pumice, added dry to mixed seed 8 days before sowing.
5. Inoculum grown in pumice, added dry to mixed seed 1½ days before sowing.
6. Inoculum grown in pumice, added dry to mixed seed just before sowing.

Four replicates of each treatment were sown, together with uninoculated control plots. The sowing was made on 21 March 1954, and the plots were then protected from birds and rabbits by wire netting placed on a light frame over the plots.

Estimates were obtained of the numbers of bacteria added per square foot in the different treatments, and these are given together with the percentages of vigorous plants obtained in counts on 29 April and 28 June 1954, in Table 4. At the second count, reliable figures were obtained only with the subterranean clover, owing to losses of white clover plants due to frost heaving.

TABLE 4. VIGOROUS PLANTS AS PERCENTAGE OF TOTAL PLANTS PRESENT AT FIRST COUNT

Treatment	Approx. No. of Bacteria per Sq. Ft.	White Clover 29/4/54	Sub. Clover 29/4/54	Sub. Clover 28/6/54
Control	—	5.5	1.5	17.5
Milk, clover only	3×10^5	43.0	30.0	60.5
Milk, mixed seed	6×10^5	50.5	27.5	55.5
Peat, dry	2×10^6	35.5	21.0	61.0
Pumice, dry, immediate	2×10^7	59.5	57.5	65.0
Pumice, dry, 1½ days	2×10^7	59.0	55.5	66.0
Pumice, dry, 8 days	6×16^6	69.5	46.5	67.0
S.E. of means		±6.4	±5.8	±7.6
Sig. diff. at 5%		19.2	17.4	

The results show that all treatments were significantly better than the control, and that mixing the pumice inoculum with the seed up to eight days before sowing had no adverse effect. The results from the different methods of inoculation are similar to those obtained from the larger plot sowings of Experiment 2, though there are some differences in the order of the treatments. However, it can be said that small plot trials on subsoil pumice are a good indication as to what is likely to be obtained with inocula or methods of inoculation when used in larger-scale sowings.

DISCUSSION OF INOCULATION EXPERIMENTS

The experiments described above show that failure of the clover seedlings to nodulate is the cause of the check to their growth, which usually occurs in pasture sowings in virgin pumice soils. This failure to nodulate is apparently due to there being insufficient numbers of clover rhizobia in the soil. Methods of inoculation suitable for use on a large scale with seed already mixed have been shown to be fully effective in promoting early nodulation of a high proportion of the clover seedlings.

In order to get a high percentage of nodulated clover seedlings, a high rate of inoculation is required. Experience in other countries with rates of inoculation is varied, but rates of the same order as found necessary here have been found an advantage in certain instances. Thus Thornton (1929), in a field trial with lucerne, found that numbers of

nodules were increased with increasing rates of rhizobia up to 20,000 per seed. Spencer (1950) obtained markedly better nodulation using massive inoculation in a pot trial in an acid soil from the South Tablelands of New South Wales. Jenkins, Vincent, and Waters (1954) found in a field trial with an acid red soil at Lismore, New South Wales, that increasing the rate of inoculation from 3,000 to 30,000 bacteria per seed increased the percentage of nodulated plants at 10 weeks from 26% to 51%. However, in contrast to this, Vincent (1954) quotes results in another soil more favourable to rhizobia where rates of inoculation varying from 300 to 30,000 bacteria per seed gave no consistent difference in proportion of nodules due to the inoculated strain.

The Australian workers (Spencer 1950; Vincent and Waters 1954) found that liming of acid soils improved them as a medium for the survival and multiplication of the rhizobia. Lime is not normally applied to pumice soil in New Zealand, for, though the calcium status is low, liming has not been found to increase pasture growth appreciably (Smallfield 1933). Although no comprehensive trial on the effect of lime on the early growth and nodulation of clovers has been carried out in the experiments here reported, indications are that no very marked effect is obtained, though a definite response in the seedling growth of lucerne was obtained in one observation trial. It is possible that lime placed in the drill-rows might improve nodulation and thus allow for a decrease in the rate of inoculum applied.

The most effective methods of inoculating mixed seed on a large scale were the application of (a) inoculum grown on finely divided peat or pumice, or (b) inoculum suspended in milk and added at about 3% by weight of the seed, both followed by thorough mixing. The peat method is the more convenient from the point of view of storage of the inoculum and application in the field. Peat inocula have been used extensively and successfully in Europe and America, though in the application of the inoculum to the seed a wet method is generally used. Recently, difficulties have been experienced in Australia (Brockwell 1954; Waters 1954) in ensuring that the peat inocula contained adequate numbers of effective rhizobia. Whether similar difficulties are likely to be serious in this country remains to be seen. Obviously, close bacteriological control is required in the preparation and storage of the inocula.

Although in Experiment 3 it was found satisfactory to mix the inoculum with the seed 8 days before sowing, this cannot be recommended at present, as later experiments indicate that delayed sowing may not always be successful. The most convenient system would be for the inoculation of the seed to be carried out in the seed store, but the indications are that this is not a reliable method, for, although under certain conditions it may be possible to retain adequate viability of the rhizobia when mixed with seed, there are many factors that may adversely affect this.

In sowing virgin pumice soils to pasture, inoculation combined with roller-drill sowing can be expected to give the following benefits:

(1) Inoculation would eliminate the early check to growth and high mortality of clover seedlings, resulting in a much quicker pasture establishment.

(2) With the uniform sowing obtained with the roller drill and the high survival rate of seedlings likely with inoculation, a reduction in the rate of seeding should be possible without adversely affecting pasture establishment, for the usual annual pasture weeds are not a problem in virgin pumice country. This saving should more than cover the cost of the inoculum.

(3) Inoculation would ensure that a strain of *Rhizobium* of proved effectiveness could be introduced.

(4) Inoculation should make possible a better early balance of clover species, with a greater proportion of white clover (the more valuable pasture species for fixing nitrogen), rather than the dominance of red clover, which is normal in the early seasons of a newly established pasture.

The reason for the greater initial survival of red than of white clover that occurs when a pasture mixture is sown without inoculation is not certain. From examination of nodules it does not appear to be due to a greater compatability of red clover than white clover with the rhizobia present in the soil. However, with subterranean clover a greater proportion of the rhizobia present are apparently ineffective, and inoculation is of prime importance for the successful establishment of this clover species.

The success of clover inoculation in virgin pumice soils opens up the possibility of its use elsewhere in New Zealand, both in the sowing of virgin soils, and also for the laying down to pasture of land that has been cropped for a number of years.

Section 2: Fertilizer and Seeding Rates

The marked effect of inoculation in improving the seedling growth of the clovers in virgin pumice soils has been described and the advantages of the roller drill over broadcast sowing briefly discussed. In order to secure the greatest advantage from these changes in sowing technique it was felt that other aspects should be investigated. In particular, information was required on the following two points:

(1) Rate and placement of fertilizer application. Would smaller quantities of phosphate fertilizer than the 3 cwt. per acre normally applied at sowing be adequate to allow for the establishment and growth over the first few months of the pasture seedlings—particularly the clover seedlings—if the fertilizer was concentrated along the drill rows, as in the roller-drill sowing?

(2) Rate of seeding. Taking into account the more even sowing and better conditions for germination obtained with the roller drill compared with broadcast sowing, together with the greater survival of clover seedlings when inoculated, would lower rates of sowing, either of the grass alone, or both grass and clover, be possible without retarding the establishment of the pasture? In sowings on virgin pumice country, the normal pasture weeds are not a problem, and some openness of the pasture in the early stages is thus not a disadvantage, and may even benefit the clovers in their establishment.

EXPERIMENTAL

Three main trials were carried out in this series, all sown in March 1954, to gain information on the above and related points.

(1) A small-plot trial on pumice subsoil, with white, red, and subterranean clovers sown with and without grass, with and without added nitrogen, and with several different phosphate treatments.

(2) A large-plot trial with a normal pasture mixture sown on cultivated pumice, with several different phosphate treatments.

(3) A large-plot trial as (2) with varying rates of seed mixtures but constant phosphate levels.

Experiment 4

A series of small plots was sown on 21 March 1954 in the experimental area near Wairakei on subsoil pumice, the methods of soil preparation and sowing being the same as described in Section 1 of this paper. Two drill rows each of white clover, red clover, and subterranean clover were sown in each plot, all seed being inoculated. There were two grass treatments, the clover seed being sown either without grass (−G), or with perennial ryegrass at approximately 30 lb./acre (+G); two nitrogen treatments, no nitrogen (−N), and high nitrogen added as urea (+N); and five phosphate treatments 0, ¾, 1½, and 3 cwt. per acre of serpentine superphosphate in the drill rows, and 3 cwt. per acre of serpentine superphosphate broadcast on the surface after sowing (P0, P¾, P1½, P3, P3b). All combinations of these treatments were included, and the plots were arranged in a split-split-plot layout with 4 replications, phosphate levels being the ultimate split and grass the primary split. Included in each block of the layout were two additional plots, both with grass, without added nitrogen, and with 3 cwt. per acre of serpentine superphosphate along the drill rows. In the first, lime was added also, at a rate of 25 cwt. per acre worked into the top 2 in. of the plot plus 4 cwt. along the drill rows. In the other about 3 in. of the pumice was removed and replaced with screened Palmerston North soil (Manawatu silt loam) which is known to support good pasture growth; the soil was then covered thinly with pumice to make the surface conditions similar to those of the other plots.

In order to minimize any adverse effect of urea on germination in the plots with added nitrogen, and yet maintain as far as possible ample supplies of available nitrogen throughout the growth period, several applications were made. Urea at 1 cwt. per acre was broadcast after sowing; then on 30 April, and again on 3 July, further urea at 2 cwt. per acre was watered along the rows.

In this experiment sown on pumice subsoil, the various phosphate treatments covered a range of levels of drilled phosphate for comparison with the standard method of 3 cwt. of superphosphate broadcast after sowing, both at an extremely low nitrogen level as well as with ample nitrogen available. The plus grass and minus grass treatments were included to determine the effect of grass competition on clover seedling growth under the different nutrient treatments. The lime plots were to test whether lime was likely to benefit seedling growth under these

conditions; and the Manawatu soil plots were included to determine if and how far the best fertilizer treatment (P3 +N) fell short in rate of seedling growth of that on a soil known to give good seedling establishment.

After sowing, the plots were protected from rabbits and birds by wire netting placed on a light frame over the plots.

Two harvests were made. One row of each species was harvested from each plot on 29 April ($5\frac{1}{2}$ weeks from sowing), and the remaining row on 1 September ($23\frac{1}{2}$ weeks from sowing). Mean plant weights

TABLE 5. MEAN PLANT WEIGHTS (MG.) OF CLOVER (TOPS ONLY) EXP. 4

Treatment N	G	P	First Harvest 29/4/54 White Clover	Red Clover	Sub Clover	Second Harvest 1/9/54 White Clover	Red Clover	Sub Clover
+	+	3	2.05	6.70	17.3	8.8	35.5	146
+	+	$1\frac{1}{2}$	2.20	6.78	15.8	12.5	29.0	79
+	+	$\frac{3}{4}$	1.73	5.43	15.8	6.3	26.5	78
+	+	0	1.08	3.60	10.8	—	1.0	7
+	+	3b	1.38	4.30	11.3	3.8	12.5	71
+	—	3	2.18	8.55	14.8	25.3	75.0	378
+	—	$1\frac{1}{2}$	2.03	7.23	14.8	27.5	30.5	203
+	—	$\frac{3}{4}$	1.68	6.03	14.3	7.3	32.5	102
+	—	0	1.00	3.33	9.3	—	—	—
+	—	3b	1.28	4.43	7.3	3.5	16.0	61
—	+	3	1.23	4.35	16.8	15.0	23.5	107
—	+	$1\frac{1}{2}$	1.10	4.13	12.8	8.3	20.0	73
—	+	$\frac{3}{4}$	1.10	4.35	12.0	6.0	17.0	73
—	+	0	0.78	3.23	10.0	—	4.5	17
—	+	3b	0.85	3.28	10.8	4.0	7.0	47
—	—	3	1.30	3.83	14.8	14.8	30.0	190
—	—	$1\frac{1}{2}$	1.35	4.03	12.3	10.0	18.5	114
—	—	$\frac{3}{4}$	1.10	4.03	11.3	4.3	14.0	55
—	—	0	0.83	3.20	7.5	—	3.5	25
—	—	3b	1.05	3.55	10.0	3.0	10.5	48
—	+	3 Lime	1.35	5.20	15.0	16.5	29.5	138
Manawatu soil			2.60	10.98	25.8	47.5	121.5	519
S.E. of means			±0.13	±0.32	±1.2	±2.7	±5.5	±46
Sig. diff. at 5%			0.40	0.92	3.5	7.9	15.7	131
F ratios from analysis of variance								
N			142.1**	53.0**	17.8*	14.9*	27.5*	5.3†
G			0.1	1.5	4.8†	11.1*	13.4**	5.5†
P			26.1**	44.4**	20.2**	20.2**	20.0**	8.2**
N × G			1.2	4.3†	0.3	13.0*	8.8*	1.7
N × P			4.7**	17.9**	2.2†	3.2*	3.4*	0.9
G × P			0.1	0.8	0.3	3.4*	3.9*	2.8†
N × G × P			0.5	2.7*	0.4	2.0	1.9	0.6

** Significant at the 1% level
* " " " 5% "
† " " " 10% "

(tops only) were determined for the three clovers and the ryegrass, in the different treatments, and are given together with results of the analyses of variance in Tables 5 and 6. The two additional treatments were omitted from all analyses.

The coefficients of variation were higher in the second harvest than in the first, partly because of damage by frost and caterpillars, the variability being greatest with subterranean clover. In the P0 treatment, insufficient white clover seedlings survived to allow of mean weights being obtained, and not many more red or subterranean clover plants survived.

Although there were differences between the three clover species, in most cases the mean weights followed a similar pattern. From Table 5 it is seen that there were highly significant differences between the phosphate treatments with all clover species at all harvests, with significant increases from nitrogen in all but subterranean clover in the second harvest. Responses to increasing phosphate were greater in the presence of nitrogen. In the first harvest with either red or white clover, in the combined nitrogen and grass means there were significant increases (indicated by $<$) in plant weight between the phosphate treatments as follows:

$$P0 < P3b < P\tfrac{3}{4} < P1\tfrac{1}{2}, P3$$

The increase $P\frac{3}{4} < P1\frac{1}{2}$ was shown in the $+N$ treatments but not in the $-N$ treatments. With subterranean clover significant increases were as follows:

$$P0, P3b < P\tfrac{3}{4}, P1\tfrac{1}{2} < P3$$

The final increase $P1\frac{1}{2} < P3$ is doubtful, being significant only in the $-N$ treatment, an unexpected result.

A definite effect from grass was shown only in red clover, grass competition giving a depression in red clover growth only at the highest phosphate level with nitrogen ($P3 +G +N$) $<$ ($P3 -G +N$). With subterranean clover no explanation can be offered for the almost significant benefit from grass, a slight increase being shown in the presence of grass in the means of all treatments.

In the second harvest, greater variability between replicates obscured some of the effects, especially in subterranean clover, but compared with the first harvest the following points are of note:

(1) Except in red clover, in the combined means of the grass and nitrogen treatments, the P3b treatment was no longer significantly lower than the $P\frac{3}{4}$ treatment, but was still significantly lower than $P1\frac{1}{2}$ treatment.

(2) With red and subterranean clover, in the combined means of the grass and nitrogen treatments there was a significant increase $P1\frac{1}{2} < P3$, this being significant in the individual comparisons in the ($-G +N$) treatments.

(3) The depression due to grass competition was clearly shown in the high phosphate $+N$ treatments in red and white clover. With subterranean clover the figures suggested a similar depression, but the analysis of variance showed this to be significant at the 10% level only.

The lime treatment in no case showed significant benefit compared with the corresponding treatment without lime (P3 +G −N). The Manawatu soil treatment is best compared with the (P3 +G +N) treatment since a certain amount of available N was present in the soil. It is seen that the Manawatu soil treatment was significantly better in all instances.

TABLE 6. MEAN PLANT WEIGHTS (MG.) OF RYEGRASS (TOPS ONLY) EXP. 4

Treatment	Perennial Ryegrass	
	First Harvest 29/4/54	Second Harvest 1/9/54
With nitrogen P3	11.1	232
P1½	11.1	158
P¾	7.9	80
P0	2.8	8†
P3b	4.3	69
Manawatu soil	17.9	106
S.E. of means	±0.9	±11
Sig. diff. at 5%	2.7	35.5
Without nitrogen P3	2.3	5.5
P1½	1.9	4.3
P¾	1.9	5.3
P0	1.5	3.8
P3b	1.7	7.0
P3 + lime	3.4	10.8
S.E. of means	±0.1	±2.3
Sig. diff at 5%	0.4	

†This treatment excluded from analysis

The results on perennial ryegrass are shown in Table 6. There was a very marked effect from both phosphate and nitrogen additions, with very little growth when either of these nutrients was not added. Thus, little information can be obtained from the −N treatment means, where nitrogen deficiency severely limited growth. In the +N treatments significant differences between the phosphate treatments in the two harvests were as follows:—

1st harvest: P0, P3b < $P\frac{3}{4}$ < $P1\frac{1}{2}$, P3

2nd harvest: P0 < P3b, $P\frac{3}{4}$ < $P1\frac{1}{2}$ < P3

It is seen that in the second harvest the P3b treatment was no longer significantly worse than the $P\frac{3}{4}$ treatment, though still behind the $P1\frac{1}{2}$ treatment. Also in the second harvest there was a significant increase from $P1\frac{1}{2}$ to P3. Owing to the extreme nitrogen deficiency, no definite conclusions can be drawn from the apparent increase due to lime. With the Manawatu soil there was a significant increase in the first harvest. In the second harvest the low value in the Manawatu soil plots appeared to be due to nitrogen deficiency, the vigorous grass growth presumably having used up the available nitrogen in the comparatively small amount of soil placed in the plots.

Experiment 5

A series of large plots was sown at Tauhara on 18 March 1954 using the roller drill described earlier. The seed mixture consisted of ryegrasses (20 lb.), cocksfoot (6 lb.), and crested dogstail (3 lb.), red clover (3 lb.), white clover (3 lb.), and subterranean clover (2 lb.); this mixture was sown at 32 lb. per acre. The mixed seed was thoroughly inoculated, both inoculum suspended in milk and inoculum grown on pumice being mixed in with the seed. Since sufficient serpentine superphosphate was not available for this trial, cobaltized straight superphosphate was used. Four replicates of the following fertilizer treatments were put down in a randomized block layout.

2.5 cwt./ac. super. drilled with the seed from separate fertilizer box.

1.6 cwt./ac. super. drilled with the seed from separate fertilizer box.

0.9 cwt./ac. super. drilled with the seed from separate fertilizer box.

3 cwt./ac. super. broadcast by hand before drilling seeed.

3 cwt./ac. super. broadcast by hand after drilling seed.

No phosphate application.

Conditions up to and after emergence were as described on p. 121. Samples were harvested on 30 April and on 1 July 1954 for determination of plant weight. The weights per plant (tops only) for ryegrass and crested dogstail taken together, cocksfoot, and white clover, at both harvests, are given in Table 7. Owing to the small numbers of red clover and subterranean clover plants harvested, the mean weights of these species lacked precision and, as they gave no information not given by white clover, they are omitted from Table 7.

TABLE 7. MEAN PLANT (TOPS ONLY) WEIGHTS (MG.) EXP. 5

Phosphate Treatment	First Harvest 30/4/54			Second Harvest 1/7/54		
	Ryegrass + Dogstail	Cocks-foot	White Clover	Ryegrass + Dogstail	Cocks-foot	White Clover
2.5 cwt./ac. drilled	7.30	2.94	1.76	27.2	9.8	5.05
1.6 cwt./ac. drilled	5.66	2.04	1.51	25.6	9.0	3.85
0.9 cwt./ac. drilled	5.16	1.89	1.30	23.0	9.7	2.95
No phosphate	2.70	1.51	1.44	5.4	4.1	1.15
3 cwt./ac. broadcast before	6.60	1.95	1.54	29.2	10.6	3.60
3 cwt./ac. broadcast after	6.02	2.26	1.65	23.0	11.7	2.20
S.E. of means	±0.42	±0.14	±0.12	±2.7	±0.6	±0.37
Sig. diff. at 5%	1.19	0.41		8.1	1.9	1.13

In the first harvest the grasses were not severely limited in growth by lack of nitrogen, and thus some differences due to the different phosphate treatments were shown up. With the grasses there was a highly significant increase in growth with increasing rate of drilled superphosphate up to the maximum used (2.5 cwt. per acre). With cocksfoot there was a significant increase due to drilled superphosphate at 2.5 cwt./acre over either of the broadcast treatments; with ryegrass plus dogstail, a significant increase compared with the broadcast after sowing treatment only.

In the first harvest of white clover, there were no significant differences. The plants were still very small and presumably still largely supported by their seed reserves of phosphate.

By the time of the second harvest the grasses appeared to be much limited by nitrogen deficiency, and the main significant phosphate effect which showed up was between the no phosphate and all of the + phosphate treatments. With white clover, although growth was poor compared with that of the other trials sown at the same time, definite differences were shown between the phosphate treatments. There was a significant increase in plant weight with increasing rate of application of drilled superphosphate up to the highest rate used. With the two broadcast treatments, 3 cwt. per acre broadcast before sowing was significantly poorer than superphosphate drilled at the same rate; 3 cwt. broadcast after sowing was significantly worse than the same dressing broadcast before sowing, and also significantly worse than 1.6 cwt. per acre drilled with the seed.

Experiment 6

This experiment, comparing different rates of seed sowing, was put down at Tauhara on 18 March 1954, the methods of sowing being as in Experiment 5. Serpentine superphosphate at 3 cwt. per acre was drilled with the seed from a separate fertilizer box in all plots. The same pasture mixture was used as in Experiment 5, and the different rates of sowing were obtained by diluting the seed mixture with grass seed previously killed by autoclaving, and sowing all plots with the same machine setting. All seed, except for the uninoculated treatment, was thoroughly inoculated by inoculum suspended in milk together with inoculum grown on pumice. The uninoculated plots were sown first, the roller drill having been cleaned out thoroughly and those parts coming in contact with the seed flushed with alcohol on the previous day after sowing the inoculated treatments of a previous trial. The treatments per acre were: not inoculated—32 lb; inoculated—32 lb., 24 lb., 16 lb., and 8 lb.

Four replicates were sown of these five treatments, set out in a randomized block arrangement, each plot being 7 ft. by 270 ft.

Throughout the winter and spring the plots with the 32 lb./ac. uninoculated treatment could be clearly distinguished by the poor growth and lack of vigour of their clovers. The 8 lb./ac. inoculated sowing was definitely open in growth though the grass plants did make somewhat more growth before exhausting their nitrogen supply, and the clover seedlings in this treatment, though few in numbers, were healthy and vigorous.

The whole experimental area received a normal topdressing of 3 cwt./ac. superphosphate in November 1954. In March 1955, one year after sowing, herbage weights of the plots were obtained with a mower and samples of this herbage were taken for botanical analysis. Also, counts were made of the numbers of surviving clover plants, six two-foot lengths of drill row being counted in each plot. The results, together with the results of the analysis of variance, are given in Table 8.

TABLE 8. MEAN WEIGHTS OF HERBAGE (LB./ACRE), PERCENTAGE OF WHITE AND RED CLOVER, AND NUMBERS OF SURVIVING WHITE AND RED CLOVER PLANTS PER 12 FT. OF ROW, IN EXP. 6 ONE YEAR AFTER SOWING

Treatment	Fresh Weight of Herbage	Percentage of White Clover	Percentage of Red Clover	No. of White Clover Plants	No. of Red Clover Plants
8 lb./ac. inoculated	910	22.6	13.1	23.3	3.0
16 lb./ac. inoculated	960	22.0	16.2	28.0	5.8
24 lb./ac. inoculated	950	23.2	16.9	29.0	5.3
32 lb./ac. inoculated	1145	27.4	20.5	40.3	8.0
32 lb./ac. uninoculated	750	16.3	15.6	17.5	3.5
S.E. of means	±40	±1.1	±1.9	±3.2	±1.0
Significance of F ratios	**	**	—	**	*
Sig. diff. at 5%	124	3.5		9.8	2.9

From Table 8 it is seen that in the fresh weight of herbage, percentage of white clover in the herbage, and number of surviving white clover plants, the 32 lb./ac. inoculated sowing gave significantly higher values than any other treatment. The 32 lb./ac. uninoculated sowing was significantly lower than any other treatment in fresh weight of herbage and percentage of white clover plants, and significantly lower than all but the 8 lb./ac. inoculated sowing in number of surviving white clover plants. With red clover the difference due to inoculation was less. The results were not significantly different in the percentage of red clover in the herbage, and in the number of red clover plants, only the 32 lb./ac. inoculated sowing was significantly higher than the 32 lb./ac. uninoculated sowing. There were no significant differences among the 8, 16, and 24 lb./ac. inoculated sowings in any of the estimations.

Experiment 7

The growth of the clover seedlings in Experiment 5 appeared to be poorer than that of corresponding treatments in the other experiments at Tauhara although no statistical test could be made of this effect. The effect did not appear to be related to the proportion of nodulated plants, as the majority of the clover seedlings in Experiment 5 were nodulated, but rather to the size and vigour of growth of the seedlings. The only difference between Experiment 5 as a whole and the other experiments at Tauhara was that in Experiment 5 cobaltized straight superphosphate was used instead of serpentine superphosphate.

A small plot experiment was then put down on subsoil pumice at Wairakei to test straight superphosphate against serpentine superphosphate. Six pairs of plots were sown with inoculated white clover and ryegrass seed, one plot in each pair having 3 cwt. per acre of straight superphosphate, and the other 3 cwt. per acre of serpentine superphosphate. The plots were sown on 17 November 1954, and half of each plot was dug up on 18 January 1955 for determinations of nodulation and weights of seedlings. Almost all the seedlings were nodulated.

There was a non-significant difference in favour of the straight superphosphate in the mean weights per plant of the nodulated clover seedlings (means 29.3 mg. and 25.1 mg., S.E. $\pm$ 1.8), and no significant differences in numbers of surviving clover seedlings or mean weights of the ryegrass seedlings.

DISCUSSION ON FERTILIZER AND SEEDING-RATE TRIALS

Superphosphate drilled with the seed at the rate of 1 cwt. per acre was equal in effect to 3 cwt. broadcast after sowing. Additional responses were obtained to heavier applications of fertilizer through the drill up to 2.5 cwt. per acre, but the smaller amount was adequate to ensure good establishment of the pasture. The smaller dressings are, of course, more convenient to apply through the drill because filling of the fertilizer box more often than the seed box is avoided.

In the field, superphosphate drilled with the seed had no adverse effect on inoculation. The moisture conditions were, however, favourable, and it remains to be determined whether serpentine superphosphate would be better under drier conditions.

It had been considered possible that clover seedlings were suppressed by the associated grass seedlings. Where superphosphate and inoculum were applied there was no evidence that the clovers suffered from competition by the sown grasses except where heavy applications of nitrogen were also made.

Although the growth of grasses and clovers was healthy and vigorous on this pumice heavily fertilized with phosphate and nitrogen, it was still more abundant on the plots of silt loam transferred from the Manawatu. The reason for this was not ascertained, but part of the effect might be due to the infrequent heavy nitrogen applications to the pumice being not as effective as the sustained supply from the loam.

Yields of the pastures from different rates of seeding at Tauhara one year after they were laid down showed that the highest rate, 32 lb. per acre, was best, but that 8 lb. of seed per acre with inoculation, produced a better pasture than 32 lb. without inoculation. It is concluded that where inoculated seed is sown with a roller drill, better pasture establishment can be obtained with less fertilizer and seed. This does not, of course, mean that there is any evidence from these trials to dispute the need for heavy dressings of phosphate for the total establishment period and for the ensuing years of pasture growth. Our main concern at this stage is the fact that smaller amounts of phosphate are satisfactory for the initial period, if drilled with the seed.

General Discussion

The investigations described in the previous sections should be considered in the light of past and present achievements in grassing virgin pumice country. Since Smallfield's (1933) original demonstration of a successful technique, hundreds of thousands of acres have been brought into pasture production as evidenced by the agricultural statistics of the region. Many thousands of sheep, dairy cows, and cattle are being carried on land which 30 years ago was waste. The emphasis in the present investigation was therefore on improving the efficiency of pasture establishment techniques and not on working out methods whereby scrub could be replaced by pasture.

Certain clear-cut conclusions can be drawn from this series of experiments. The roller drill, with its capacity to form steep-sided channels which automatically concentrate seed and fertilizer in discrete bands at optimum depth below the surface, appears to give better emergence and certainly gives more efficient utilization of fertilizer during the establishment phase. Seed and fertilizer placement, however, does not overcome the high mortality of clover seedlings, which in turn seriously delays the raising of soil nitrogen to the level where a balanced grass—clover association can be maintained. Marked improvement in the number of emerged clover seedlings which grow into healthy well nodulated plants was achieved by various inoculation techniques used in conjunction with the roller drill. This is seen particularly clearly in Table 8, which shows that a sowing of 8 lb. per acre of inoculated seed was significantly better than 32 lb. not inoculated, both sown with the roller drill. It is not suggested that such a low seeding rate is desirable, but when, as at present, seed prices are high, it is obvious that considerable economies can be effected by marked reduction in seeding rates. Further work is desirable on the proportion of the different species making up the seeds mixture, but the results from the 8 lb. per acre sowing show how important is the saving that can be achieved. If the results can be applied to full scale operations, this saving should more than counter-balance the cost of inoculation and whatever extra charges are incurred in changes in equipment.

The adoption of roller drilling plus inoculation of the seed should soon be practicable on the ploughable land, for the roller drill is now being manufactured in New Zealand and in Australia, and inoculum for clovers is becoming available in larger quantities. The integration of the necessary changes in technique into the present large-scale sowing operations should not present insuperable difficulties.

From the success with which pasture was established both on subsoil, and on the difficult soil type at Tauhara, the desirability of better levelling of the areas during development assumes added importance. At present, every endeavour is made to retain an even covering of topsoil. From the results of the field plot comparisons between topsoil and subsoil, it appears that the major advantages of the topsoil lie in its sparse population of rhizobia and slight initial supply of nitrogen, both topsoil and subsoil being otherwise very poor in plant nutrients. If, in fact, the differences between topsoil and subsoil can be removed by inoculation and adequate phosphate applications, there remains no valid reason against land levelling during the developmental programme prior to sowing. At that stage there are no fences, and

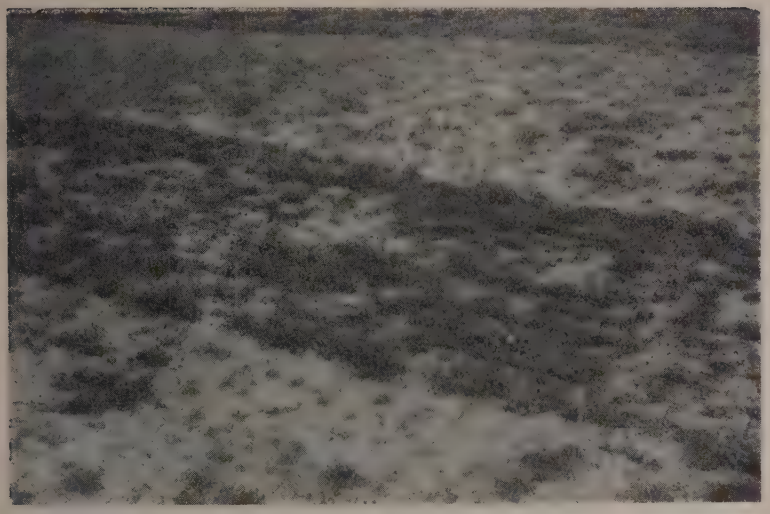

Fig. 10.—Comparison between broadcast sowings (foreground and third from front) and sowings with the roller-drill, all seed inoculated. Photograph taken in March 1955 near experimental area at Tauhara.

heavy machinery can undoubtedly be used more economically on large blocks than later by the individual farmer.

Finally, the technique used in the field trials reported in this paper, i.e., the use of the shaped board to simulate a roller drill, and the discarding of the patchy topsoil as a means of obtaining a uniform sample of the soil of lowest fertility, have both proved very helpful in clarifying the early experimental studies. It is of interest to note that this technique is proving of value in further studies on this and other areas.

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SEED DISINFECTION

XI. CONTROL OF HEAD SMUT (*Sorosporium reilianum* (Kuhn) McAlpine) OF MAIZE

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Summary

In field trials, dusting maize seed with materials based on organic mercury, dichlone, and thiram, gave significant reduction of head smut (*Sorosporium reilianum* (Kuhn) McAlpine) of maize.

Introduction

A survey of maize (*Zea mays* L.) crops carried out in 1952 in the Gisborne district disclosed a range of from 0% to 22.4% of plants infected with head smut (*Sorosporium reilianum* (Kuhn) McAlpine). The highest incidence occured on areas where maize had been grown for 8 or more successive seasons, the lowest in freshly broken pasture land. The district average was 2.9% infection.

Infected plants were found either in groups or in isolated positions scattered over a field. Previous observations (Jacks, 1951) have shown that early infection became apparent in a number of isolated plants widely dispersed over a crop and was spread by spore dispersion during harvesting operations.

Soil fumigants (Jacks, 1951) are uneconomical for control of head smut. To investigate less costly control methods, various seed dressings were used in trials conducted over a period of three years. Results are reported in this paper.

Materials, Methods, and Results

Certified maize seed of the variety Pfister 360 was used in all tests.

The following seed dressings were used: Agrimax M (4% phenyl mercuric dinaphthyl methane disulphonate); Agrosan GN (1% tolyl mercury acetate); Anticarie SD (11.2% polychlorobenzene); Barbak (8% mercuric phenyl cyanamide); copper 8 quinolinolate; Cuprox (50% copper oxychloride); Fernasan (50% thiram); Panogen (0.8% methyl mercury dicyan diamide); Phygon XL (50% dichlone); Spergon (98% chloranil); Tetroc (98% chloranil); Thirodust (75% thiram); Zinc 8 quinolinolate; 8 quinolinol; 8 quinolinol benzoate; 8 quinolinol sulphate; and 36L (98% 1-(p-sulphamylphenyl)-3 : 5-dimethyl-4-nitrosopyrazole).

Materials were applied in a rotary shaker at rates given in the tables. Where concentrations exceeded the amounts naturally taken up by seed, a methyl alcohol resin sticker was used to obtain greater adhesion. All seed was treated with 25% lindane at 0.2% of seed weight to prevent wireworm injury to seed and seedlings.

Formalin (40% formaldehyde) and technically pure paraformaldehyde were used in addition to the above materials as Dillon Weston and Schofield (1952) reported control of millet smut (*Sphacelotheca destruens* (Schlecht. Stevenson and Johnson by seed treatment with a number of compounds including formaldehyde. However, maize seed treated with Formalin and paraformaldehyde, applied at 0.2% of seed weight, failed to emerge.

A lindane bait prepared with bran and molasses was distributed in the field tests to prevent losses of seedlings caused by cutworm.

Experimental methods followed closely those reported earlier (Jacks, 1951).

Glasshouse emergence tests were carried out in boxes containing approximately 0.5 cu. ft. of steam-disinfected soil. Fifty seeds were sown in each box, there being 7 replications per treatment. Emergence records taken three weeks after sowing are given in Table 1.

TABLE 1. EMERGENCE OF MAIZE SEEDLINGS IN THREE GLASSHOUSE TESTS

Treatments	Treated at Percentage Rates of Seed Weight					
	0.2%		0.4%		2.0%	
	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle
Check — untreated	80.3	63.7	80.3	63.7	80.3	63.7
„ + wetting agent	78.4	62.4	78.4	62.4	78.4	62.4
„ + 25% lindane	80.4	64.0	80.4	64.0	80.4	64.0
Agrimax M	73.9	59.3	74.9	60.0	67.3	55.1
Agrosan GN	82.3	65.2	76.2	60.9	82.7	65.5
Anticarie SD	82.3	65.2	85.1	67.4	79.5	63.1
Barbak	93.3	75.4	83.2	65.9	77.6	61.8
Copper 8 quinolinolate	88.6	70.5	76.5	61.3	73.7	59.2
Cuprox	94.4	76.7	87.7	69.7	82.7	65.4
Fernasan	94.4	76.4	91.8	73.4	92.0	74.2
Panogen	90.9	73.0	78.3	62.4	57.8	49.5
Phygon XL	85.5	67.9	80.5	63.8	80.3	63.9
Spergon	92.7	74.4	88.1	70.1	75.7	60.6
Tetroc	89.9	72.2	87.1	69.1	83.0	65.9
Thirodust	89.7	71.3	79.1	63.1	80.5	63.9
Zinc 8 quinolinolate	88.8	70.5	89.4	71.5	75.3	60.3
8 quinolinol	90.6	72.6	77.0	61.4	73.2	58.9
8 „ benzoate	72.5	58.4	74.1	59.4	69.8	56.7
8 „ sulphate	74.4	59.7	74.5	59.7	77.7	61.9
36 L	87.0	69.7	77.7	62.2	82.4	65.3
Difference required for significance at 1%		5.1	—	4.5	—	4.1

Emergence was increased by Fernasan at all concentrations tested and less consistently by Cuprox, Spergon, Tetroc, zinc 8 quinolinolate, Barbak, copper 8 quinolinolate, Panogen, Thirodust, 8 quinolinol, and 36L. The following materials depressed emergence: 8 quinolinol benzoate consistently, and Agrimax M, copper 8 quinolinolate, Panogen, and 8 quinolinol at the highest dosage level under test.

In glasshouse tests, carried out with inoculated seed and soil (Jacks, 1951), seed treatments failed to give consistent reduction in the number of infected plants. However, the number of plant replicates (12) was insufficient to allow for adequate evaluation of treatment efficacy.

Field experiments were carried out to check the effect of treatments on emergence and on incidence of infection. In Auckland, replicates consisted of single rows 11 ft. in length sown 1 ft. apart and randomized in blocks. Fifty seeds were sown per row and there were 8 replications per treatment. Records were taken 3 weeks after emergence.

In Gisborne, replicates consisted of 4 rows sown 35 in. apart running the length of fields used for trials. There were 8 replications

TABLE 2. EMERGENCE OF MAIZE SEEDLINGS IN FIELD TRIALS

Treatments	Auckland		Gisborne					
	1950*		1950/51*		1951/52*		1952/53**	
	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle
Check	80.4	64.1	80.4	64.3	55.6	48.2	70.9	57.4
Agrimax M	83.3	66.3	—	—	60.4	51.1	—	—
Agrosan GN	85.0	67.9	75.0	60.5	63.0	52.6	77.9	62.1
Anticarie SD	78.1	62.8	84.0	67.1	—	—	—	—
Barbak	84.3	67.4	77.9	62.4	—	—	—	—
Cuprox	67.6	55.5	81.2	66.5	—	—	—	—
Fernasan	77.1	62.0	81.7	65.6	—	—	—	—
Panogen	82.3	65.6	91.3	75.3	—	—	—	—
Phygon XL	88.7	71.8	85.6	69.6	56.2	48.6	76.3	61.1
Spergon	86.1	63.7	—	—	—	—	—	—
Tetroc	77.3	62.1	82.4	67.8	—	—	—	—
Thirodust	84.6	67.4	—	—	74.9	60.1	76.5	61.2
36L	84.7	67.3	80.0	63.8	—	—	—	—
Difference required for significance at 1%	8.1		—	11.8	—	4.8	—	5.5

* Applied at 0.2% of seed weight

** „ „ 0.4% „ „ „

per treatment randomized in blocks. Approximately 13 lb. of seed were sown per acre (about 20,500 seeds). Recording was made 6 weeks after sowing from the two centre rows of each replicate, counts being made from 10 lengths of 10 ft. taken 20 to 30 ft. apart.

Lack of facilities prevented the continued testing of the full range of materials in the Gisborne field trials.

Results of field emergence records are given in Table 2.

Although in one instance Thirodust gave significant increase and Cuprox significant depression in emergence, results, in general, showed that seed treatments did not materially affect emergence under conditions of these tests.

Records of head smut incidence from Gisborne crops shown in Table 3 were made 2 to 3 weeks before harvest in a manner similar to that of emergence counts.

A significant increase in the final stand of plants was recorded in the following treatments: Agrosan GN, Agrimax M, Phygon XL, and

TABLE 3. FINAL STAND OF MAIZE IN GISBORNE FIELD TRIALS WITH PERCENTAGES INFECTED PLANTS

Treatments	Healthy Plants†						Infected Plants‡					
	1950/51*		1951/52*		1952/53**		1950/51*		1951/52*		1952/53**	
	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle
Check	46.8	43.1	50.1	45.0	59.7	50.7	2.2	8.5	6.0	13.8	3.4	10.4
Agrimax M	—	—	59.4	50.5	—	—	—	—	3.7	10.8	—	—
Agrosan GN	54.2	47.7	57.5	49.3	68.8	56.1	1.5	5.8	3.8	10.8	1.0	5.6
Anticarie SD	57.3	49.3	—	—	—	—	1.0	4.2	—	—	—	—
Barbak	51.2	45.7	—	—	—	—	1.2	5.7	—	—	—	—
Cuprox	57.6	49.5	—	—	—	—	2.0	8.0	—	—		—
Fernasan	55.8	48.4	—	—	—	—	1.0	5.1	—	—	—	—
Panogen	57.2	49.2	—	—	—	—	1.9	7.6	—	—	—	—
Phygon XL	55.8	48.6	53.9	47.2	68.6	56.0	1.1	4.6	3.5	10.4	1.4	6.4
Tetroc	59.1	50.4	—	—	—	—	1.7	7.2	—	—	—	—
Thirodust	—	—	66.9	55.0	65.0	53.7	—	—	3.1	9.8	1.0	5.0
36L	57.2	49.3	—	—	—	—	1.6	7.0	—	—	—	—
Difference required for significance at 1%		8.6	—	3.0	—	4.8	—	4.1	—	2.3	—	2.7

† Calculated from seeding rate * Applied at 0.2% of seed weight

‡ „ „ percentage stand ** „ „ 0.4% „ „ „

Thirodust. Significant reduction of head smut in the first trial was brought about by Anticarie SD. In the last 2 trials Agrosan GN, Phygon XL, and Thirodust, gave significant reduction of disease incidence without being significantly different from each other.

CONCLUSION

Where crop rotation cannot be practised, seed treatment is an inexpensive way of diminishing losses due to head smut, as the amount of chemical used is very small in relation to the quantity of seed. The lowering of disease incidence in the field indicates that seed treatment of maize with organic mercury (Agrosan), thiram (Thirodust), or dichlone (Phygon XL), can be recommended.

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EFFECT OF TREATMENT OF DAIRY PASTURES WITH BHC AND DDT ON THE FLAVOUR AND COMPOSITION OF MILK, CREAM, AND BUTTER

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Summary

Treatment of dry closely grazed pastures with 2 lb. of lindane or with 2 lb. of pp'-DDT per acre, with a 3-week interval before grazing, did not cause taint in milk, cream, or butter. The butter contained 4-6 parts per million of benzene hexachloride and no pp'-DDT. Treatment under similar conditions with BHC (mixed isomers) at the same rate of dosage of gamma isomer as for lindane caused a slight taint in the milk, cream, and butter, and the butter contained 16-18 p.p.m. of BHC.

Treatment of long pastures, when wet, at the same rate of dosage, and grazing of the area immediately, gave (a) with lindane a "chemical taint" in the milk, cream, and butter, and a rapid rise in the BHC content of the butter; (b) with BHC (mixed isomers) a strong "earthy" or "musty" taint in the milk, cream, and butter, and a rapid rise in the BHC content of the butter; (c) with DDT a "sweetish" but not objectionable taint in the milk and cream but not in the butter, and a rapid rise in the DDT content of the butter. The "chemical" and "earthy" taints from the lindane and technical BHC treatments persisted in the milk for some time after removal of the cows from the treated areas. It is concluded that if pastures are treated strictly according to the recommended procedure there is no likelihood of New Zealand factory-made cheese and butter containing an appreciable proportion of insecticides. Because of its lower tainting properties DDT appears to be the most suitable of the insecticides for the treatment of dairy pastures.

Introduction

Treatment of pastures with benzene hexachloride (BHC) or with DDT insecticide has been recommended by Dumbleton, Kelsey, and Hoy[1] and Kelsey[2] for control of the common grass-grub (*Costelytra zealandica* (White)) and the subterranean caterpillar (*Oxycanus* sp.). It is probable that the insecticide treatment, hitherto mainly confined to sheep-grazing pastures, will soon be extended to dairy pastures. The present investigation has been undertaken to determine (a) whether treatment of pastures according to the recommended procedure[2] will cause taint in dairy products made from the milk of cows grazing on the areas; and (b) the effect on quality of dairy products of a considerable divergence from the recommended procedure.

Benzene hexachloride is a variable mixture of isomers of hexachlorocyclohexane with small amounts of other substances, e.g., heptachlorocyclohexane and chlorophenols, and it has an odour which is usually described as "fusty" or "earthy". The isomers differ in their odour characteristics. The gamma isomer, which occurs to the extent of 12% to 13% in commercial BHC and which is the only strongly insecticidal isomer, has only a faint odour scarcely evident at a concentration of 1 part in 100,000[3]. The epsilon isomer, on the other hand, can be detected by smell in a solution containing only 1 part in 1,000,000,000.

Lindane is the common name for benzene hexachloride containing not less than 99% gamma isomer. Kirsch and Schwarz[3] found a solution containing 1 part per 100,000 of lindane to be distinctly musty in odour, and a solution containing 1 part per 20,000 to be very musty.

There is obviously a greater risk of causing taint with BHC (mixed isomers) than with lindane; but even lindane may vary in composition, i.e., within the 1% limit of impurities it may contain a variable proportion of the more odorous isomers and related compounds. Results obtained with any one commercial BHC preparation may not apply exactly for other BHC preparations, and results obtained with one preparation of lindane may not be applicable with other preparations. Thus the results quoted below, both those extracted from the literature and those in the present report, may not apply in detail for all preparations of lindane or technical BHC commercially available.

DDT is a mixture of the two isomeric dichloro-diphenyl-trichloroethanes. The para para isomer (p.p.i.) is the active constituent. Commercial DDT preparations, which usually contain a proporttion of filler dust, are evaluated on the p.p.i. present in the powder as sold. Pure DDT has a somewhat sweetish taste but is much less odorous than BHC preparations.

Overseas Investigations

It is well established that cows sprayed with chlorinated hydrocarbon insecticidal substances[4 to 9], or given fodder sprayed or dusted with these[10 to 21], for control of infestations of various kinds, secrete some of the insecticide into the milk or the body tissues. Because of their insolubility in water and their ready solubility in fat the insecticides become concentrated in the milk fat or in the body depot fat. When cream is separated from contaminated milk the insecticide is concentrated into the cream by the fat, and when the cream is churned almost all of the insecticide is found in the butter. Mann and Carter[10], for example, found 7.5 p.p.m. of DDT in a raw whole milk containing 5.0% of fat, 67.2 p.p.m. in the 70% cream from this milk and 0.2 p.p.m. in the skim milk, 100 p.p.m. in butter from the cream and 1.9 p.p.m. in the buttermilk. There was no loss of DDT during the processing.

Insecticides stored in the body fat are gradually released, with the result that their excretion in the milk may continue for some time after ingestion of them has been discontinued.

The effect on the flavour of the milk naturally varies according to the organoleptic properties of the pure insecticidal substance and to the degree of purity of the insecticidal preparation used, as stated above. The relationship between quantity of insecticide ingested and quantity appearing in the milk has not been fully explored, but with cows under similar lactation conditions, and with approximately similar yields of milk and butterfat, the concentration in the milk is roughly proportional to the concentration in the feed.

The following brief survey of the literature will serve to indicate the nature of the results already available.

Benzene Hexachloride Preparations

Furman and Hoskins[11] reported that cows given a single dose of BHC (mixed isomers) at the rate of 40 mg/kg body weight gave milk

with a "musty" taint for 10 days. The milk contained 5 p.p.m. of gamma BHC. They reported that a definite odour was noted in cream containing 1 p.p.m. of gamma isomer representing a content of 30 p.p.m. of BHC. Kaestli and Gutknecht[12] stated that spreading of BHC preparations on grassland, if the insecticide adheres to the grass, causes a characteristic smell and taste in the milk. Kirsch and Schwarz[3] fed 20 g of lindane to a cow and found no recognizable effect on the flavour of the milk. When they increased the daily intake to 30 g there was a distinct deterioration of the taste of the milk but the odour was not affected. Kaegi[13, 14] concluded that the milk of cows given fodder soiled with BHC preparations was not consumable. Ely *et al.*[15, 16] fed lindane to cows at various rates of dosage and found the content of the insecticide in the milk to be roughly proportional to the amount ingested. Some of their results are shown in Table 1. Ten days after

TABLE 1. EFFECT OF FEEDING LINDANE TO COW FOR 70 DAYS ON BHC CONTENT OF MILK (after Ely *et al.*[16])

Weight Fed Daily (mg)	Mg. kg of Body Weight	p.p.m. in Diet	Weight Excreted in Milk (mg)	p.p.m. in Fat-corrected Milk
25	0.07	2.3	0.93	0.07
50	0.15	4.9	1.36	0.11
250	0.67	23.1	3.14	0.39
1000	2.95	95.6	28.7	2.69

discontinuance of the lindane feeding the concentration of BHC in the milk was reduced to half that present during the feeding period, and it decreased by a further 50% during each of the two succeeding 10-day periods. At the lower dosage levels of 25-125 mg daily, a more or less characteristic flavour was frequently detected and the averaged flavour readings were in the same order as for the dosage level. The flavour was described as "stale", "bitter", "musty", "cowy" or, simply, "off-flavour". At the higher dosage levels Ely *et al.*[15] found the flavour caused by lindane feeding to be more pronounced in the butter than in the milk. In butters from the milk of cows receiving 100, 125, and 150 mg of lindane daily, the taint could be detected only by the experienced grader, but in butter from cows receiving 1000-2000 mg daily the taint could be detected with certainty by inexperienced graders.

DDT Preparations

Allen, Lardy and Wilson[17] fed DDT-treated silage (1 lb. of insecticide per ton) to cows, at the rate of 3 lb. per 100 lb. live weight daily, for 118 to 140 days. The milk near the close of the experimental period contained 15.6 p.p.m. of DDT. The muscle tissue of slaughtered animals contained 3.8 p.p.m. and the depot fat 221 p.p.m. The milk of other animals in the group held for 1 month after discontinuance of feeding of DDT-treated fodder contained 7 p.p.m., and the depot fat 38 p.p.m., of DDT. Wilson[18] fed silage containing 500 p.p.m. of DDT to cows, so that the average level in the diet was 60 to 100 p.p.m., for 5 months. He found 1.15 p.p.m. of DDT in the milk and 300 p.p.m. in the body fat. Biddulph *et al.*[19] fed hay dusted with 36 p.p.m. of

technical DDT to 16 Holstein cows over 2 years. Some DDT appeared in the milk after 4 days and the amount gradually increased until a fairly constant level was reached. When feeding of the treated hay was discontinued there was a rapid fall in DDT content of the milk, but some DDT was being excreted in the milk 4 months later. During the trial, up to 7.5 p.p.m. of DDT was found in the milk, but there was no evidence of any fault in flavour. Shepherd *et al.*[20] fed cows on hay from pastures treated with 2.4 lb. DDT per acre and reported that with a daily intake of 903 mg DDT the amount found in the milk was 265 mg per day. The output in the milk varied from 5% to 30% of the intake and was roughly proportional to the intake. Oral intake of large amounts resulted in persistence of DDT in the milk for periods of up to 170 days, and of small amounts for periods of 30 to 40 days. Ely, Moore, Carter, Mann, and Poos[21] found higher concentrations of DDT in the milk when the insecticide was given as a residue in field-sprayed alfalfa than when it was administered at comparable dosage rates as crystalline DDT. Increase in dosage rate of DDT gave a corresponding increase in the amount of DDT excreted in the milk.

Summarizing the literature, it is clear that when insecticide is present in the feed to any appreciable extent a portion of it is excreted in the milk. BHC (mixed isomers) may cause a "musty" or "earthy" taint, lindane a much less distinct "fusty" taint, and DDT has little effect on the flavour of milk and its derivatives.

Experimental

The work reported here was carried out at The Dairy Research Institute (N.Z.), Palmerston North, during October-December, 1952. In Trial No. 1 the insecticides were applied strictly according to the recommendations of Kelsey[2], viz.:

1. The insecticide-containing superphosphate or lime should be applied to pastures when they are close-grazed and dry.
2. If it is not possible to wait for close-grazed pastures to dry, stock should be kept off the area after treatment until rain has washed the insecticide on to the ground.
3. The insecticide mixture should be broadcast, not drilled in.

Since, however, at times it is difficult to obtain a uniform close-grazing of dairy pastures unless sheep are available also on the farm, and since the treatment may be applied by some farmers without sufficient attention being given to the recommendations, it seemed necessary to investigate the effect of treatment under less satisfactory conditions. For Trials 2 and 3, therefore, the conditions of treatment were chosen to represent what was considered to be the maximum divergence from the recommended procedure likely to be encountered in practice. For these trials the application was made on damp long pasture, early in the morning while the dew was still on the foliage, and the cows were grazed on the area immediately after the insecticides were applied.

In all the trials the plots were break-grazed, i.e., the cows were given access each day to only the proportion of the area judged adequate for a day's ration.

METHODS OF ANALYSIS

The content of pp'-DDT in the butter was determined by the method of Schechter and Haller[22] after the fat had been removed on a column as in the modification of Davidow[23]. The content of BHC was determined by the method of Schechter and Hornstein[24]. This method gives the total amount of BHC present, and does not measure the content of the gamma isomer separately.

Blank determinations on control butters from cows grazing on untreated areas were variable and higher than expected. (With the analytical methods used it was not possible to distinguish between the insecticides and certain related substances.) Care was taken to avoid contamination of the control plot by drift during treatment of the experimental areas, but there may have been some transfer by wind after treatment, to account for the high control values recorded in Table 4. The experimental results obtained are given in the tables below, i.e., corrections for the values obtained on the control samples have not been made.

DETAILS OF TREATMENT, AND RESULTS

Trial No. 1

The insecticide was applied on close-grazed pastures, and the pastures were not grazed until after a considerable amount of rain had fallen. A 2-acre paddock that had previously been cut for silage was used. The aftermath had been kept down by grazing, with the result that the pasture was short and open. The paddock was divided into 4 half-acre plots by means of an electric fence. The control plot was treated with superphosphate only, and the other three were treated at the rate of 2 lb.-equivalent of insecticide active isomer per acre, in admixture with superphosphate in accordance with the recommendations of Kelsey[2].

The insecticides used were (a) *lindane* in the form of a 20% dust at 10 lb. per acre, (b) a *commercial BHC preparation* containing 10% of gamma isomer, at 20 lb. per acre, and (c) *DDT* in the form of a wettable powder containing 50% of pp'-DDT, at 4 lb. per acre. After the treatment on 6 November 1952 the area was closed to stock until 1 December, when the grazing of the pastures was commenced. During the interim there had been rain on 20 out of the 24 days, with falls of 0.26, 0.51, and 1.75 in. on the three days immediately subsequent to treatment, and a total fall of 6.92 in. for the period. On the two days after the beginning of grazing there were 0.54 and 0.84 in. of rain. It is clear, therefore, that the conditions approximated closely to the requirements set down by Kelsey[2].

At the beginning of December, when grazing was begun, the area was covered with vigorous white-clover-dominant pasture. A cow was grazed on each of the three treated plots and one cow on the control plot. Milk yields of all cows fell during the first day of the trial, probably owing to the disturbing effect of the change from herd grazing to isolated grazing. They were normal during the remainder of the 18-day sampling period. There were no changes in percentage fat content of the milk or in yield of butterfat that could be attributed to the effect of the insecticides.

The milk and cream from the cow grazed on the plot treated with technical BHC was tainted on several occasions, but only to a slight extent. Milk, cream, and butter from the cows on the lindane- and DDT-treated plots were free from taint throughout. From the insecticide contents of the butters analysed (Table 2) the following conclusions may be drawn:

TABLE 2. Insecticide Contents of Butters (Trial 1)

Time after Commencement of Grazing (days)	Benzene Hexachloride (p.p.m.)			DDT (p.p.m.)	
	Control Area	Lindane-treated Area	Technical BHC-treated Area	Control Area	DDT Area
1	3	7	12	2.1	0.8
2	2	7	14	2.4	1.2
3	3.2	7.5	21	1.0	1.7
4	3.0	11	21	3.7	3.8
12	3.0	9	19	1.3	0.2

(a) Even after treatment of the pasture under the very favourable conditions described above there was ingestion of BHC from the lindane-treated area in sufficient quantity to cause some excretion of BHC in the milk and therefore a significant content of BHC in the butter. The rate of excretion had increased to a maximum after 3 days and persisted for the whole 12-day period of grazing for which butter analyses are available.

(b) Treatment of the area with technical BHC caused a higher rate of excretion of BHC than treatment with lindane, no doubt because of the higher rate of treatment required to provide the desired dosage of gamma isomer. In some of the butters containing up to 20 p.p.m. BHC, presence of an insecticide taint was not observed by the graders.

(c) There was no evidence of ingestion of DDT in sufficient quantities to cause significant excretion of the insecticide in the milk.

Trial No. 2

BHC insecticides were applied to long wet pastures. The three one-acre plots used had a thick covering of ryegrass and white clover about 10 to 12 in. long. The control plot was treated with superphosphate alone. The lindane and technical BHC were applied between 7 and 8 a.m. when the pasture was wet with a heavy dew that caused the applied mixture to adhere to the foliage. The odour of the insecticide was strongly evident in the neighbourhood of the treated areas. There were 0.31 in. of rain during the first 24 hours, 0.20 in. during the next 24 hours, 0.05 in. during the third 24 hours, and 0.30 in. during the ninth 24-hour period. During the remainder of the trial there was no rain.

Trial 2A. Two cows were placed on each plot immediately after completion of the treatment (as stated above, they were given access to only a portion of the area each day).

Trial 2B. After four days, one cow from each of the three plots was transferred to untreated pasture. The milks were sampled at intervals and examined to provide evidence of the extent to which the taint persisted when the cows were no longer ingesting the insecticide.

Trial 2C. As a check on the extent to which the effect on the treated pastures persisted, a cow was placed on each of the treated plots 15 days after the date of treatment.

Trial 2A

Lindane. A slight taint was evident in the milk and cream from the two cows on this plot at the first milking, and it remained evident during the 18-day period of grazing of the animals on the plot. The taint was described by the graders as a "chemical" flavour.

Technical BHC. A slight taint was observed in the milk and cream at the first milking, and a strong taint at the second and subsequent milkings throughout the whole period of grazing of the cows on the treated plot, i.e., for 18 days. The taint in the cream was described by the graders as "earthy" or "strong musty". When cream showing the strong taint was diluted 1 in 30 with clean pasteurized cream, the taint was still evident to the sense of smell. When it was diluted 1 in 60 it was evident to the taste only.

Since, at the time of the trial, facilities were not available for analysis of the insecticide content of the dairy products, a full range of samples of butter was not retained. A few samples, however, were held for later analysis. The results (Table 3) confirm the findings of

TABLE 3. BHC Content (p.p.m.) of Butter (Trial 2A)

Days after Treatment	Control Area		Lindane-treated Area		Technical-BHC-treated Area	
	Cow A	Cow B	Cow C	Cow D	Cow E	Cow F
Bulked sample for first two days	6.4	6.1	33	113	825	271

the organoleptic examination, that there was a rapid transfer of insecticide from the pasture into the milk and the butter. Within two days the BHC content of the butter from one cow on the technical-BHC-treated area had reached nearly 0.1%. The daily yields of milk from the cows were within the range 30 to 45 pounds. There was no difference in either yield or fat content of the milks from cows C and D or from cows E and F, to account for the difference in insecticide contents of the butters as shown in Table 3.

Trial 2B

In the cream and butter from the cow removed from the lindane-treated area and grazed on uncontaminated pasture the lindane taint was still noticeable after seven days, but it was no longer present after a further three days of normal grazing. Both cream and butter from the cow removed from the technical-BHC-treated area was still strongly tainted ten days later, when sampling was discontinued.

Trial 2C

The areas used were portions of those treated as described for Trial 2A and still not grazed by stock. The total rainfall during the interim was 0.56 in. and there was a further fall of 0.30 in. on the first day of grazing. The odour of the insecticide was still readily observable above the pasture. In the cream and butter from the cow on the lindane-treated area there was no taint on the first day of grazing but a definite taint on the second day. In the cream and butter from the cow on the technical-BHC-treated area there was a slight taint on the first day and a strong taint on the second day of grazing.

Trial No. 3

Long wet pastures were treated with DDT (as for Trial 2 with lindane and BHC), and were grazed immediately. There was no rain during the period when the cows were grazing on the area.

Two cows were used, one on a control area and one on the treated area. The milk and cream from the cow on the treated area had a foreign flavour described by the grader as "sweet, but not seriously objectionable". This taint appeared at the first milking and persisted throughout the three days of the trial. It was not noticeable in the butters.

TABLE 4. DDT Content (p.p.m.) of Butters (Trial 3)

Duration of Grazing on Area (days)	Control	Experimental
1	4.4	32
2	4.6	157
3	7.8	183

The results for DDT content of the butters (Table 4) show that the excretion of DDT in the milk was considerable after one day's grazing and had risen almost to the maximum after two days' grazing.

Discussion

Permitted limits for pesticides are fixed in U.S.A., Great Britain, and New Zealand, only for fruit and vegetables. The limit for DDT in the three countries is 7 p.p.m. A limit for BHC has been set only in U.S.A., where it is 5 p.p.m. for BHC (mixed isomers) or 10 p.p.m. for lindane.[25]

Milk delivered to cheese factories, or to dried-milk and casein factories where it is factory-separated, and farm-separated cream delivered to butter factories, are bulked in large vats at not less than two stages during the processing to the final dairy product. The milk or cream from any one farm thus becomes incorporated with a large volume of milk or cream from other farms. This has an important bearing on any consideration of the possible effect of insecticidal treatment of pastures on the insecticide content of commercial dairy products.

The present investigation has shown that treatment of pastures with lindane according to the recommended procedure and at the highest recommended dosage can cause the appearance of some BHC *in an individual farm butter*, but the content would not be above the limit set in U.S.A. for vegetables and fruit. It is unlikely therefore that with careful treatment of the pastures with lindane excessive amounts of BHC would be found in commercial butter, which is drawn from a large supply area. Because of its tainting properties and the higher dosage rate necessary to give the required amount of gamma isomer per acre, BHC (mixed isomers) should not be used for the treatment of dairy pastures. DDT applied according to the recommended procedure gives no taint and the DDT content of the butter was not greater than in the control samples. DDT therefore is the most suitable of the three insecticides for the treatment of dairy pastures.

Under the very adverse conditions adopted for Trial 2A there was with technical BHC, and to a lesser extent with lindane and DDT, tainting of the milk, cream, and butter, and a sharp increase in the insecticide content of the "individual-farm" butter. It is scarcely conceivable that these conditions would apply even on one supply-farm; but, should this happen, the contamination would be observed by the sense of smell in the milk or cream from this farm on the factory receiving-stage.

It is concluded that DDT and lindane can safely be applied to dairy pastures. DDT is preferable to lindane because of the somewhat greater tainting properties of lindane when applied under unsatisfactory conditions (compare Trial 2A with Trial 3). The results from Trials 2 and 3, however, indicate that careful control of the conditions of application is essential. The recommendations of Kelsey[2] should be strictly followed.

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STUDIES IN USE OF AGRICULTURAL LIMESTONE

II. SOLUBILITY OF LIMESTONE IN ACID SOIL AS INFLUENCED BY PARTICLE SIZE

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Summary

1. In 50 years over 80 papers have been published in English detailing experiments on the effects of fineness and hardness on the solubility* of agricultural limestone. The conflicting and frequently inconclusive nature of the results is shown to be due to differences in the conditions and methods of experiment. Even when confined to direct measurements of carbonate solution, comparisons made in the earlier diverging parts of the solution curves are shown to have enhanced the apparent differences between limes and finenesses, while those made on the later converging parts of the curves minimized the differences.
2. Some previous workers have postulated an equal rate of diameter reduction affecting all particles alike, but their published experimental data did not confirm this for carbonate particles in the soil. When examined in this light, however, many of the previously published solution data are shown to conform to this rule.
3. Studies on the solution in a moist acid soil of 25 South Island (N.Z.) limestones, at known finenesses, are described, and progressive solution curves presented. Particle size has a far greater influence on solubility than do the physical properties of the stone. This paper considers the effects of fineness only. Solution is shown to adhere closely to the rule of equal diameter reduction in the case of the coarser particles, but to deviate from the rule as fineness increases. The deviation, which is appreciable only in the earlier stages of solution, is of small practical consequence, and predictions based on the rule are thereafter highly successful.
4. This simple situation applies only to carbonate particles intimately mixed at modest rates with a moist and sufficiently acid soil. Evidence is accruing to show that increasing the lime:soil ratio, as by heavier application, indifferent mixing, or surface application, suppresses the soil's dissolving power, the solution of the finer particles being affected first.
5. The conclusions drawn in paragraph 1 (above) are confirmed on theoretical grounds and it is concluded that no statements can be made on the relative solubility of agricultural limestones that do not make *some* assumptions as to the period of time involved and the dissolving power of the soil.

Introduction

Ever since the practice of burning lime for agricultural use gave way to the crushing of the limestone itself, the questions of how finely the stone should be crushed and of the relative degrees of pulverization required by different types of limestone have been subjects of scientific enquiry. During the past 50 years, no fewer than 80 papers have been published (counting only those in the English language) detailing investigations on this apparently simple problem. That such repetitious enquiry was due to the inconclusive nature of the results being obtained is confirmed by the fact that in 1940 the Agricultural Lime Investigational Committee[1] found it necessary to recommend that still further

*The terms "soluble" and "solubility" are used here in the sense of ease or rate of solution. No equilibrium between solid and its solution is implied.

research be instituted on the relative merits of hard and soft limes, and fine and coarse grinding. The previous studies on these questions have been well reviewed from time to time, especially by Pierre[2], Lyon[3], Walker, Brown, and Young[4], and Fippin[5], and it is not necessary to repeat that procedure here. However, they have varied widely in the methods employed and in the conclusions drawn, and it is necessary to comment on certain aspects. Various-sized sieve separates of different limestones have been compared by three different methods: (a) by rate of solution of the carbonates in soils, or other acid media; (b) by effects on soil properties such as pH, base saturation, exchangeable H, Ca, Mg, or Mn, rate of nitrification, etc.; (c) effects on crop yield. The limes have been mixed with one or more soils in some arbitrary proportions and the results judged after equally arbitrary periods of time, ranging from a few days to several years. It has perhaps seemed more direct and practical to study the effects on soil or plant growth; but, as pointed out by Pierre[2] and Lyon[3], such comparisons are greatly influenced by the conditions of the experiment, such as type of soil and its chemical constitution, soil:lime ratio, species of crop used, weather conditions, level of natural or induced fertility, and, most important of all, the length of time over which the comparison is made. The consequence is that the results obtained and the conclusions drawn have been widely divergent. A few examples will illustrate the point. Bleacher *et al.*[6] reported that 20–40 mesh limestone applied to an acid silt loam at full lime-requirement rates, gave negligible yields of red clover and lucerne, compared with the robust growth promoted by < 100-mesh limestone. Walker[7] on the other hand, reporting on a series of field trials in Great Britain, including a variety of different soils and crops, found that 20–40 mesh limestone gave crop yields only a few per cent lower than those promoted by < 100-mesh carbonate and by burnt lime. Lyon[3] found a succession of crops responded to 5–10 mesh carbonate with yields only 20% less than those obtained by < 200 mesh limestone, whereas Rigg[8] found the > 20 mesh separate had negligible effect on the yield of beans and peas compared with that of < 60 mesh limestone. The considerable number of such empirical and apparently conflicting observations recorded in the literature, while possessing agronomic significance in the particular situations employed, do not provide a satisfactory solution to the basic problem of how finely limestones should be ground.

Since the only inherent property limiting the usefulness of limestone in the soil is the rate at which its calcium (and magnesium) can pass into solution, it would seem that carbonate solution studies offer the most logical and least subjective approach to the problem. How effective that calcium will then be depends on a host of circumstances quite independent of the nature of the limestone employed. But even within this narrower field of enquiry widely divergent conclusions have been reached. It has become clear from a study of the published data, supported fully by the experimental results reported below, that this is due to (a) differences in the period of time over which the comparisons have been made, and (b) variations in the relation between the factors fineness, type of stone, soil:lime ratio, and the exchange capacity, lime requirement, and dissolving power of the soils employed. Every one of these factors influences the ratio of the solubility of particles of one size to those of another, in some cases to a considerable degree. It will be shown that the solution curves of limestone separates differing in

fineness or hardness diverge in the earlier stages of solution and converge in the later stages; also that all curves conform closely to the same mathematical form. It follows that any factor, or combination of factors, that tends to minimize solution (such as short-term comparisons, drier or less acid soil, harder or coarser limestone) results in comparisons being made on the earlier divergent part of the curves, so that differences tend to be maximal. Conversely, any factor that favours increased solution (such as long-term comparisons, wetter or more acid soils, softer or finer limes) result in comparisons being made in the later converging part of the curves, where differences between coarse and fine, hard and soft limes are minimized. Considering the time factor, for example, all limestones, whatever their state of subdivision, are equally soluble if given sufficient time, and long term comparisons give opportunity for the slow solution of the coarser or harder limes to overtake the more rapid solution of the finer or softer limes and so to appear in a relatively more favourable light; whereas short-term comparisons discredit the coarse or the hard limes. Numerous illustrations of the above generalization occur in the literature, and the following examples are typical. Williams[9] found little difference in the solution and soil effects of separates ranging from 8–20 to < 90 mesh; a result that can be explained by the fact that his comparison was made over a reaction period of 2½ years. MacIntyre and Shaw[10] found less difference between the solution of calcitic and dolomitic limestones when their finer fractions were compared, while the greater dissolving power of a sub-surface soil horizon narrowed the difference between these limestones and between their 10–20 and 20–40 separates. Doak[11] found that the difference in solubility of hard and soft limestones, considerable with the coarser fraction, was small when the finer fractions were compared. The results of Morgan and Salter[12] show that, of the two soils employed, the more acid soil greatly reduced the differences between the solubilities of 40–50 and 80–100 mesh limestones. It is clear that differences between the rates of solution of different limestones and sizes of particle are not absolute differences, but are qualified, often to a major degree, by the conditions of their usage. Some more fundamental, less empirical, approach to the problem is needed.

Hypothesis of Equal Reduction

Such an approach has been deleveloped by Kriege[13], Bear and Allen[14], and Salter and Schollenberger[15, 16] and Schollenberger and Whittaker[23]. Kriege studied the reaction of limestone separates with buffered HCl and succinic acid solutions. Having determined the rate of solution by weight at known carbonate surfaces, he measured in the same terms the specific surface of a number of limestone separates, and demonstrated that the factor* relating mean particle diameter and total effective surface did not change materially when irregularly shaped limestone particles ranging in size from 4 to 150 mesh were considered. He found that provided no disintegration of particles occurred, a straight-line relation existed, for any one limestone, between log solution rate and log particle size. It can readily be calculated from equation 1 (below) that this corresponds to an equal rate of diameter reduction operating at all surfaces. Bear and Allen[14], discussing the soil-limestone reaction, postulated that all carbonate surfaces should be dissolving

*For any mass of material subdivided into uniform cubes or spheres, diameter × total surface = constant, irrespective of changes in diameter. The factor referred to corresponds to this constant.

at an equal rate, and that therefore (a) diameters of all particles should be reduced by equal amounts during a given period of time; (b) the rate of solution of any particle size, or composite of sizes, could be calculated from the diameter reduction of some standard particle size in a particular soil. On this basis they calculated a table of relative efficiencies for the different sieve separates, and described experiments designed to test their hypothesis. Salter and Schollenberger[15, 16] presented similar theoretical tables, based on the above supposition, using the fundamental equation

$$R = \left(\frac{D - d}{D}\right)^3 \qquad 1.$$

where R is the fractional residue (by volume or weight) remaining after particles of initial diameter D have experienced a reduction d in their diameters. From experiments on the solution of limestone separates in various soils (results apparently not published) they report that values of d varied directly with time, as expected, and inversely with the ratio of dolomite to calcite.

More recently, Schollenberger and Whittaker[23], in studies on the solution of limestone separates in boiling ammonium chloride solution, calculated theoretical solution curves based on a constant rate of diameter reduction, and found that under these conditions of solution the diameters of the smaller particles were reduced at a slower rate than those of the coarser particles, though factors were noted that would account, at least in part, for this anomaly. Their reported diameter reductions (termed *a* values) for the same limestones when embedded in soil for 18 months do not reveal the constancy of otherwise of *a* as particle size was varied.

Unfortunately the experimental results published by the six last-mentioned authors did not go far in the direction of establishing the application of this "equal reduction" hypothesis to the behaviour of limestone in the soil. However, it is possible to test the hypothesis by reference to solution data published by previous workers, in the following way. From equation 1 it is a simple matter to calculate from their initial mean diameters (D) the percentages of solution which would be experienced by the various sieve separates if all particles suffered some constant reduction in diameter, d. Plotting these percentages against log mean diameter of the separates, a smooth curve is obtained for each value of d. In Fig. 1 the solid lines are such "equal reduction" curves, corresponding to various indicated values of d. There are two ways of expressing the solution of carbonate particles: (a) by progressive diameter reduction, (b) by percentage loss in weight or volume. These curves express the relationship between the two. It follows that any series of two or more limestone separates whose observed percentage solutions, under given circumstances, are plotted in like manner, will yield points lying along a line parallel with the theoretical curves if all their particles have also suffered some constant reduction in diameter. A selection has been made from the solution data reported in the literature, of those cases in which the mean diameters of the separates used are adequately defined. A representative group of these are plotted (broken lines) in Fig. 1. The numbers attached are those of the references to the papers from which the solution data have been taken. References 13 and 20 relate to limestone separates in acid solutions; the rest to limestones in soils. It is at once apparent, from the general parallelism between the experimental and the theoretical curves, that

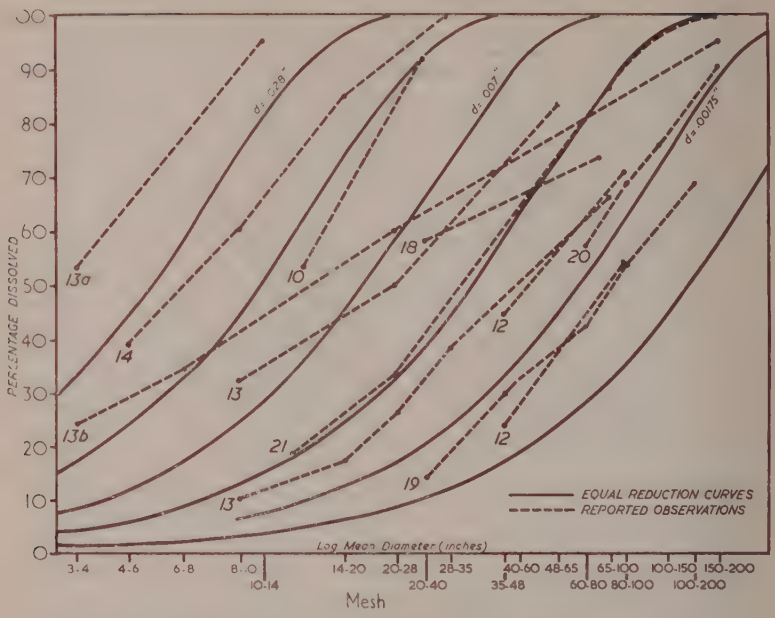

Fig. 1.—Calculated "equal reduction" curves (solid lines) show the solution percentages that would result from diameter reductions (d) equally affecting all particle sizes. Actual solution data similarly plotted (broken lines) run generally parallel to the calculated curves, conforming to the equal reduction hypothesis. The numbers relate to the references on pp. 172, 173.

the hypothesis of equal reduction receives substantial support from the previously published data. The results, seemingly so contradictory when expressed as percentages dissolved, resolve themselves into a consistent behaviour when this is expressed in terms of diameter reduction. The author's results (see Fig. 3) confirm the hypothesis.

Only three sets of results have been encountered that seriously deviate from the rule, each in the direction of coarser particles having dissolved relatively more than the theoretical expectation. (See Fig. 1, curves 13b and 18, and the results of Schollenberger and Whittaker[23] referred to above.) Kriege noted this anomaly in his own results[18] and suggested two causes: (a) extra internal surface associated with very coarse porosity, a character that is largely dissipated in the finer fractions[17]; (b) disintegration, which was observed to occur with some of the coarser particles. Support for this explanation is lent by the fact that a coarsely porous friable limestone presents the greatest deviation from theoretical expectation that has been encountered (curve 13b), whereas pure calcite in the same trial conformed to the rule (curve 13a). The exception shown by curve 18 cannot be explained on these grounds, but could be due to the more rapid neutralization of the soil by the solution of the finer particles, and consequent suppression of dissolving power. The author has obtained evidence that this does undoubtedly occur in the case of heavy applications. (Results to be reported in a subsequent paper.)

SOLUBILITY TRIAL ON N.Z. LIMESTONES

Recently Elphick[17] examined the chemical and physical properties of 32 South Island (N.Z.) agricultural limestones with a view to determining the influence exerted by those properties, together with fineness of grinding, on rate of solution in an acid soil. The experiments to that end are now described and the results examined insofar as the effects of fineness of grinding are concerned. The influence of the chemical and physical nature of the limestone in conjunction with fineness will be dealt with in a succeeding paper.

Twenty-four of the limestones, judged to include all variants of likely significance, together with one Westland limestone not included in the original survey, were selected for solution studies. It was recognized that most agricultural limestone used in New Zealand is applied as topdressing, and that the relative solubilities might not be the same under such conditions as when the limes are intimately mixed with the soil. But it was believed, correctly as it turns out, that the uniform conditions of admixture with the soil were more likely to provide the fundamental information being sought. Topdressing trials, now under way, are providing a logical development from the simple situation reported on here. Since rapidly dissolving fine limes were to be compared with slowly dissolving coarse limes over a period of six to eight months, it appeared essential to avoid the disturbing influence of seasonal changes in temperature and moisture that would be experienced out of doors. The trial was therefore conducted in the artificially standard climate of a constant temperature room; a factor which governed the technique employed. The object was to obtain progressive solution data from which the weekly rate of diameter reduction (Δ) could be calculated from the equation:

$$R = \left(\frac{D - t\Delta}{D} \right)^3 \qquad 2.$$

in which t is the period of solution in weeks.

EXPERIMENTAL PROCEDURE

All 25 limestones were tested at the one fineness, 20–40 mesh. In addition, seven of them representing the major types, were also tested at the finenesses 10–20, 40–80, and 80–100 mesh. The designation of the limestones by letters of the alphabet is the same as that employed for the description of their chemical and physical properties[17] to which reference should be made.

The Westland limestone, now included, is assigned the letter i, and may be described as a compact, very hard, brownish-grey and coarsely crystalline limestone, containing 91.1% of carbonate. The five sieve sizes, and the geometric mean diameters of the separates are given in Table 1. The four separates approximate to a logarithmic series in their mean particle diameters. The screened separates were thoroughly water-washed to remove adhering fines and to slake aggregrates, then were re-screened and air dried. Their carbonate contents were determined with the Collins calcimeter, as the same method was to be used to determine residual carbonate in the soil samples. By adopting certain refinements in the use of the calcimeter, a mean recovery of 99.8% of the carbonate present with a standard error of 0.27% was obtained. The soil used was Wakanui silt loam, 0–4 in., from an area of the College

farm that had been intensively cropped and unlimed for 12 years. The chemical data are listed in Table 1. The lime-requirement, to pH 7.0, was determined by Schofield's para-nitro-phenol method[22]. The lime separates were intimately mixed with the screened (5 mesh) and air-dried soil, at the rate of 6.0 m.e.% or 0.300% of $CaCO_3$, allowance being made for the impurities in the separates.

TABLE 1. Experimental Data

Sieves		Limestone Separates Geom. Mean Diam.		Soil Data	Temp. Variation in Storage Room (°F.)		
Mesh	Mean Aperture Inches	Mesh	Inches	Wakanui Silt Loam (0-4 in.)	Month	Mean Max.	Mean Min.
10	0.0728	10-20	0.0484	pH 5.4	July 1948	57.5	55.4
20	0.0324	20-40	0.0222	Exch. capacity 18.4 m.e.%			
40	0.0153	40-80	0.0103	Base satn. 52%	Jan. 1949	62.4	58.1
80	0.0070	80-150	0.00525	Lime requirement 8.0 m.e.%			
150	0.0039			Lime added 6.0 m.e.%			

All calculations were based on the oven-dry weight of the soil, to correct for variable drying conditions during the seven months of the trial. Ten identical mixes of each treatment (450 g. soil per mix) were prepared, each mix being placed in an open-ended metal cyclinder ($4\frac{1}{2} \times 2\frac{1}{2}$ in.) standing on a bed of sand which served to provide uniform drainage conditions. The soil surfaces were covered with a $\frac{1}{4}$ in. mulch of fine gravel, and all containers given repeated additions of rain water, using a self-filling drenching gun with spray nozzle, till drainage just commenced. Thereafter, to simulate the leaching effect of field conditions, the tins were given $\frac{3}{4}$ in. rain water each fortnight, equivalent to 20 in. of rain per annum. Owing to the negligible evaporation, this maintained the soils in a moister condition than obtains for a large part of the time in the field. It is estimated that the soils were maintained at a pF value of about 1.9. Clear evidence that leaching of calcium was taking place was derived from a limited number of pH measurements, though there was no intention of measuring leaching under these highly artificial conditions. The highest pH produced, by the rapid solution of the finest separates, was 6.7. The temperature of the room was regulated by thermostatic control, and over the period of the trial varied by a few degrees on either side of 60°F. Temperature ranges based on daily readings are included in Table 1. Sampling, at intervals varying from one to four weeks, consisted in lifting whole cylinders and quickly drying the contents. To ensure sufficiently accurate sub-sampling, it proved necessary to grind the dried soil samples till all carbonate would pass 60 mesh. This was effected in a porcelain ball-

mill. The residual carbonate content of all soil samples was determined by Collins calcimeter. To avoid bias in any form, the order of preparation of the treatments was randomized, the 460 cylinders were set out in ten randomized blocks, and the operations of withdrawal, preparation, and analysis of the samples were performed in random order. A dummy set of mixes was prepared and carbonate analysis of 27 of these (unwatered) revealed that the standard error attributable to preparation and analysis was $\pm$ 0.97% of the application.

RESULTS

The percentages of solution so recorded are presented in Table 2. The individual figures, each representing the contents of one cylinder, are at times erratic, but they serve to check and correct one another, and the derived estimates of rate of solution are freed from much of this

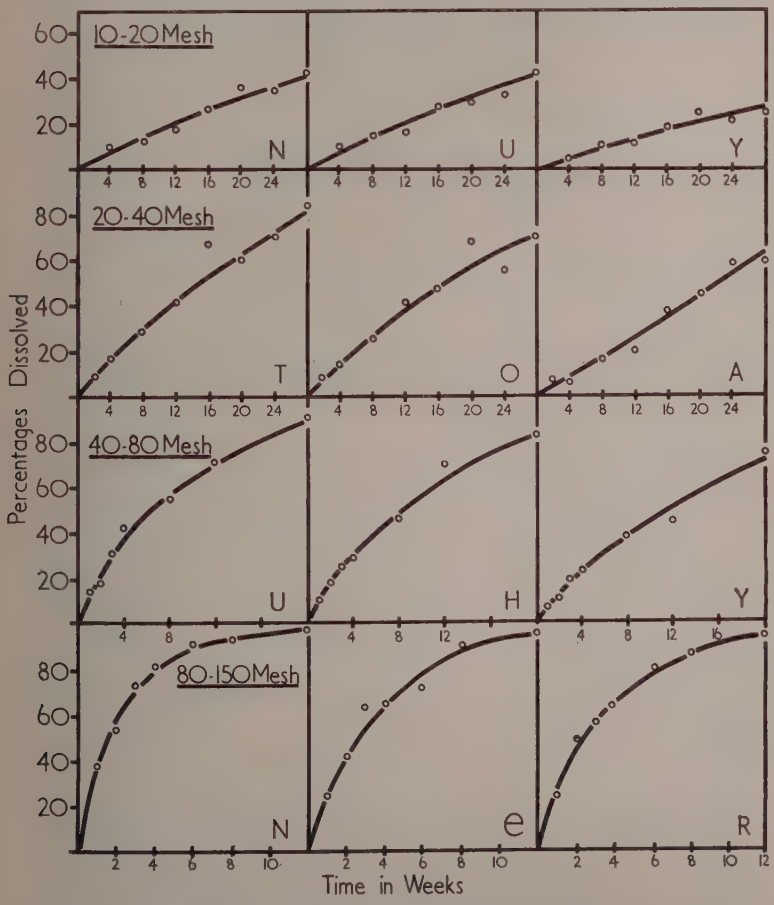

Fig. 2.—A representative selection of the 46 solution curves. Each point is based on a separate cylinder of soil. Letters relate to the designation of the limestones (See Table 2, and Ref. 17).

TABLE 2. Percentages of Carbonate Dissolved in Stated Periods, Mean Rates of Solution, and Mean Rates of Diameter Reduction (Δ)

Lime-stone	Mesh	Weeks												Mean Rate %	Δ inch × 10⁻⁴
		1	2	3	4	6	8	12	16	20	24	28	32		
H	10-20				10		18	20	24	27	32	38	35	1.06	2.31
N	,,				11		13	18	27	36	35	43	40	1.32	2.65
R	,,				6		8	15	20	29	23	25	32	0.96	1.80
T	,,				8		13	17	26	30	35	33	35	1.11	2.27
U	,,				10		15	17	28	30	33	43	44	1.47	2.65
Y	,,				5		11	12	19	25	22	25	30	1.00	1.68
e	,,				7		15	16	27	35	35	33		1.47	2.47
	Means				8.1		13.3	16.5	24.2	30.3	30.8	34.2	36.0	1.20	2.26
H	40-80	11	19	26	30		47	71		84	93			6.3	2.52
N	,,	15	26	30	42		61	67		92	98			8.6	3.05
R	,,	12	22	27	33		48	73		85	95			6.6	2.66
T	,,	11	23	29	42		50	76		88	92			8.0	2.65
U	,,	15	18	32	42		56	72		92	89			7.9	2.96
Y	,,	8	12	20	24		39	46		76	82			4.1	1.88
e	,,	10	20	21	33		60	72		84	91			7.1	2.51
	Means	11.7	20.0	26.4	35.1		51.6	68.2		85.9	91.5			6.9	2.60
H	80-150	27	52	58	63	75	84	94						21.7	2.98
N	,,	38	54	74	82	92	93	98						31.8	3.89
R	,,	25	49	57	64	80	87	95						21.3	3.14
T	,,	35	43	62	74	82	84	95						23.1	3.18
U	,,	32	48	66		89	89	98						25.0	3.55
Y	,,	24	34	53	55	69	83	95						15.6	2.88
e	,,	25	42	64	65	72	91	96						19.2	3.23
	Means	29.4	46.0	62.0	67.2	79.9	87.3	95.9						22.5	3.26

H	20-40		10			23	33	34	46	52	61		2.17	2.07
N	,,		17		17	27	37	47	64	63	85		2.94	3.15
R	,,		8		9	23	26	49	46	52	48		2.27	1.94
T	,,		10		17	30	43	68	62	72	85		3.45	3.49
U	,,		10		16	26	38	55	60	55	75		3.03	2.75
Y	,,		3		10	16	25	39	35	51			2.02	1.81
e	,,		7		14	28	39	44	58	65	69		2.94	2.66
	Means		9.3		13.8	24.7	34.5	48.0	53.0	58.5	70.5		2.69	2.55
A	20-40		8		7	17	21	38	46	59	60		2.27	2.09
C	,,		2		7	13	19	27	39	36	63		1.83	1.70
D	,,		4		10	11	24	30	45	37	41		1.72	1.51
G	,,		4		14	17	30	38	55	46	49		2.13	1.90
K	,,		9		17	34	40	56	66	63	80		3.36	3.11
L	,,		5		8	14	27	34	46	58	64		2.27	2.14
M	,,		6		11	34	37	46	68	86			3.12	3.61
O	,,		9		15	26	42	48	69	56	71		3.03	2.76
P	,,		16		24	42	58	83	78	89	91		5.00	4.73
Q	,,		8		13	20	38	46	70	55	65		2.86	2.58
W	,,		6		14	17	27	47	48	64	55		2.38	2.21
a	,,		12		15	32	34	49	56	63			2.98	2.71
b	,,		8		11	20	33	44	56	57	55		2.50	2.21
d	,,		5		13	25	38	47	49	57	51		2.74	2.14
f	,,		7		13	24	47	57	58	63	63		2.98	2.65
g	,,		7		12	30	36	54	56	66	65		2.90	2.64
h	,,		8		11	20	30	48	53	47			2.35	2.17
i	,,		10		11	16	26	34	51	49	53		2.04	1.90
	Means*		8.0		12.9	23.4	33.9	46.6	55.2	58.5	64.2		2.69	2.51

* 25 Limestones

S.E. applicable to all values of $\Delta = 0.27 \times 10^{-4}$

experimental error. As has been noted already, the progress of solution can be expressed in two ways, (a) in terms of percentage loss by weight, (b) in terms of diameter reduction. Both methods have been used to express comparative solubilities, viz, in terms of Mean Rate of solution up to the point of 50% solution (in each case) expressed as percentage dissolved per week, and in terms of Δ, the weekly rate of diameter reduction, calculated as the linear regression coefficient of the equation $t\Delta = D\ (1 - \sqrt[3]{R})$ from the listed solution data for each treatment. These two estimates are listed in Table 2. Apart from a few anomalous cases, the measure of relative solubilities provided by the two methods shows satisfactory agreement. For the series of 25 separates at 20–40 mesh they show a high correlation ($r = 0.96$).

Mean Rates were deduced from progressive solution curves constructed from the data in Table 2. A representative selection of these is presented in Fig. 2. The straightness of the curves for the coarser separates contrasts noticeably with the inflected form of the "fine" curves. As will be seen later, the "coarse" curves are the early nearly linear sections of more extended curves following the same general equation (see Fig. 5). The abnormal form of curve shown by limestone A (20–40 mesh) is shared by a small group of similar dense hard stones, and the reason for this will appear in a subsequent paper.

Comparison between limes and finenesses is most easily seen in terms of Mean Rate. This function gives expression to the greater solution of finer separates occasioned by their greater surface area. It is obvious without further demonstration that differences between the four finenesses are highly significant, and that solubility is affected to a far greater extent by fineness of grinding than it is by differences in the type of stone. Excluding the soft chalk, P, the softest limestone (20–40 mesh) is just twice as soluble as the hardest, a difference in solubility that is more than equalled by each 50% reduction in particle size. It will also be noted that in terms of Mean Rate the seven limestones selected for study at four finenesses are a truly representative group.

Differences between limes, expressed by either method, are highly significant, and will be examined in the next paper of this series. For the present, attention will be focussed on the influence of fineness.

Solution as Affected by Fineness

The support given by the results to the hypothesis of equal reduction can be demonstrated in two ways: graphically, by the comparison of experimental and theoretical curves; and, more exactly, by regression analysis of the data, the results of which will be discussed in the following section. In Fig. 3 the mean progressive solution data for the seven limestones at the four finenesses, derived from the smoothed curves, are plotted (broken lines) together with the "equal reduction" curves (solid lines) as already employed in Fig. 1. The first point to note is the striking parallelism between the experimental and the theoretical curves from 12 weeks onwards. This confirms for the major period of the experiment, (from the 12th week onwards) what has already been inferred from previously published data, namely that limestone particles of various sizes, buried in a moist acid soil, suffer equal diameter reduction at all surfaces; or, expressed in a slightly different way, solution is proportional to surface area, as would be expected. Taken individually, the curves for the seven limes deviate

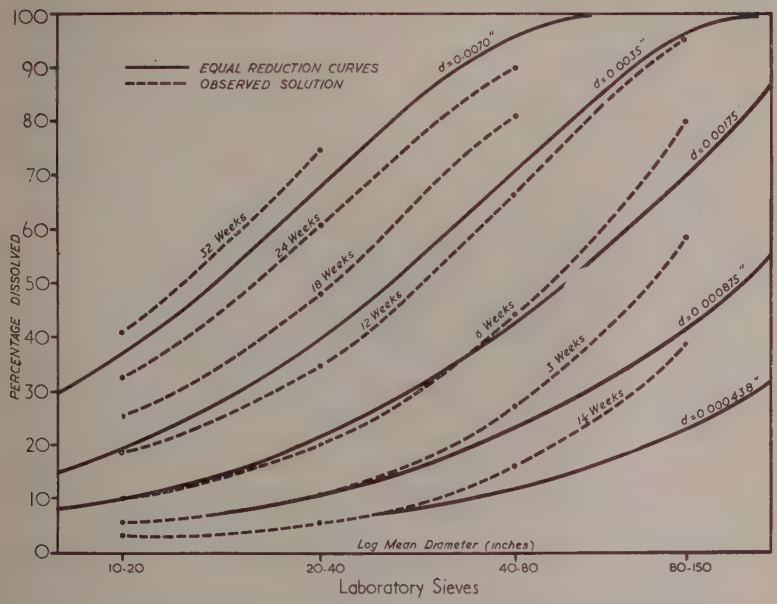

Fig. 3.—The parallelism between the curves derived from observed solution (Table 2, mean of 7 limestones) and the calculated equal reduction curves, confirms the equal reduction hypothesis. An exception is noted in respect of the early solution of the finer particles.

very little from the parallelism displayed by their means. The second notable point is that in the first one and a half weeks the two finest separates (especially the 80–150 mesh) dissolved relatively much faster at their surfaces than did the coarser separates, giving the first experimental curve ($1\frac{1}{2}$ weeks) a sharp upward tilt. Thereafter, as solution proceeds, the experimental curves gradually fall back into line with the equal reduction curves till by 12 weeks they are parallel again. This means that after the initial "spurt" displayed by the 40–80 and 80–150 separates, their rates of diameter reduction rapidly decline, and in the later stages of solution are slightly less than that of the coarse separates.

This deviation from the hypothesis is more clearly illustrated in Fig. 4, in which are shown progressive changes in rate of diameter reduction (Δ) experienced by the four separates (mean of 7 limes) calculated from the modified form of equation 2:

$$\Delta = \frac{D(1 - \sqrt[3]{R})}{t} \qquad 3.$$

Also shown in Fig. 4 are the mean values of Δ for the respective separates over the first 12-week period. All diameter reduction rates show a decline during the first few weeks. The finer the particles the more marked is this effect. Thereafter they remain fairly constant and of the same order for all finenesses. The cause of these deviations from the hypothesis will be examined in a subsequent paper. The more intensive initial action at the surface of the finer particles is thought to be attributable to a more effective association between that highly curved surface and the surrounding soil.

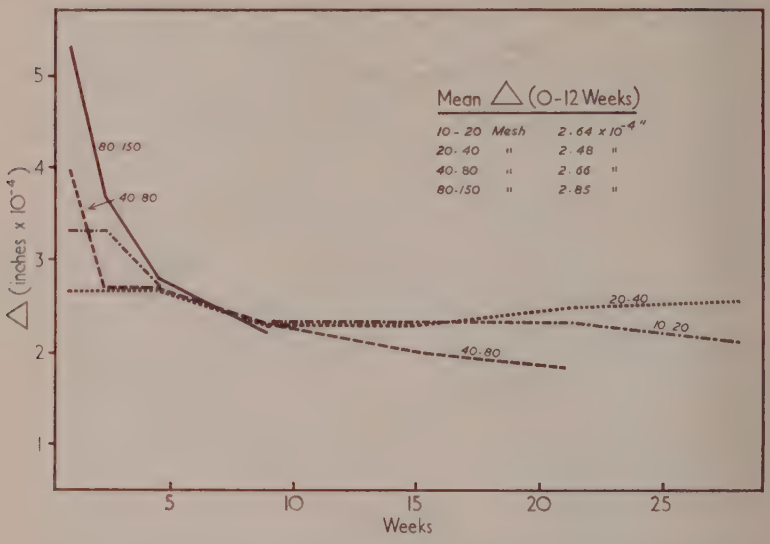

Fig. 4.—Rates of diameter reduction (Δ) in a moist acid soil of limestone particles of four sizes (sieve designations as shown) at intervals after commencement of reaction. Mean of seven limestones.

If, now, a constant value of Δ (2.5×10^{-4} in.) is assumed to apply from the commencement to all particles, progressive solution curves may be calculated from equation 2. These are shown in Fig. 5, compared with the mean experimental data for the seven limes. The observed solution of the two coarser separates coincides quite remarkably with the prediction, while the only serious discrepancy is that affecting the early solution of the 80–150 mesh material. After 12 weeks very little error is involved in asuming a constant value of Δ to have applied to all separates from the start.

Test of the Hypothesis

It will be clear from equation 3 that if the hypothesis of equal reduction were being strictly adhered to, then the function $D\ (1 - \sqrt[3]{R})$ would prove linear with respect to time for all values of D. The linearity of this function has therefore been tested by regression analysis* of the solution data recorded in Table 2. Deviations from linearity are then judged by the significance of the second degree coefficients. The results confirm the conclusions already presented. It will be noted that the mean values of Δ for the first three separates (Table 2) are strikingly close, considering the diversity in size and character of the limestone particles involved; while the higher value for the 80–150 separates is in accord with their high initial speed of reaction already noted. With regard to the second-order terms, these are non-significant for the coarsest separate, and hardly significant in the case of the 20–40

*Acknowledgement is gratefully made to the Applied Mathematics Laboratory, D.S.I.R., Wellington, for these calculations.

separates; but there is an increasing proportion of significant terms as fineness increases. Thus:

10-20 mesh separates	.....	.....	.....	No 2nd degree terms significant			
20-40 „	„	.....	4 out of 25	„	„	„	..
40-80 „	„	.....	3 out of 7	„	„	„	„
80-150 „	„	.....	6 out of 7	„	„	„	„

This fully supports the evidence already presented in Figs. 3, 4, and 5, and leads to the conclusion that the hypothesis is followed very closely by the two coarser separates, but that the deviation increases thereafter with fineness. Analysis of variance of the Δ values in Table 2 shows a high order significance (0.001) for the differences between limes, no significant interaction between limes and particle sizes when the three coarser separates only are considered, but a significant interaction when the finest separates are included. This, again, is in accord with the general conclusions.

Application

In weighing up the practical significance of the above deviations from the hypothesis of equal reduction, it will probably be agreed that in view of the customary practice of supplying substantial dressings of lime at intervals of several years, differences in the rate of solution of the rapidly dissolving finer fractions are of small practical consequence. A few weeks more or less in the time taken for their solution are of slender importance compared with the differences in calcium supply that can arise from the long-drawn-out solution of the coarser fractions of different samples. It is concluded, therefore, that when crushed limestone is intimately mixed with a moist acid soil, the rule of equal diameter reduction can *for most practical purposes* be assumed to apply to all particle sizes. In spite of the great differences in density, purity,

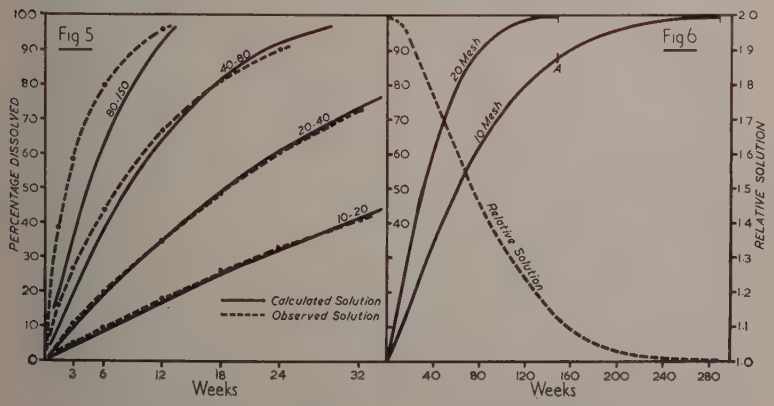

Fig. 5.—Progressive solution curves of four sieve separates in a moist acid soil: (a) observed solution, mean of 7 limestones, (b) calculated from Equation 2. ($\Delta = 2.5 \times 10^{-4}$ in.).

Fig. 6.—Calculated solution curves for limestone particles of two sizes. (Equation 2. $\Delta = 2.3 \times 10^{-4}$ in.). The ratio between their degrees of solution diminishes rapidly as solution proceeds.

porosity, and particle shape of these limestones[17], each separate has behaved in the soil as if composed of uniform spheres possessing the geometric mean diameter of that separate, just as was found by Kriege[15] to be the case with limestone separates when reacting with an acid solution.

This simple relationship enables certain deductions to be made. It was pointed out earlier that diverse conclusions as to the relative solubility of the different particle sizes had been drawn, depending on whether comparisons were made in the earlier diverging or later converging portions of the solution curves. It is now clear from the mathematical form of these curves that this must be so. Fig. 6 shows the theoretical solution curves for particles that would just pass the 10 mesh and the 20-mesh sieves respectively, calculated from equation 2, with a value of $\Delta = 2.3 \times 10^{-4}$ in. Also shown is the rapidly diminishing ratio (relative solution) between the degree of solution of these two particle sizes. Initially, by virtue of the given mass possessing twice the surface area, the finer particles dissolve at twice the rate. (Relative solution = 2.0). But the greater the initial rate the more rapidly does it slow down, and the difference between the two steadily dwindles till eventually they have become equally soluble. This is a direct consequence of the mathematical relationship between linear and cubic dimensions; but, as will be shown in the succeeding paper, differences in hardness of the stone, or in soil conditions, which likewise increase or decrease the rate of solution, impose a similar variation in the relative degrees of solution of different limes and finenesses, and it is clear, as was recognized by Schollenberger and Salter[16], that no statements can be made on the relative solubility of agricultural limestones that do not make *some* assumptions as to the period of time involved and the dissolving power of the soil.

The rate of supply of soluble calcium diminishes rapidly as solution proceeds. Although the diameter of the particles is being reduced at a constant rate, the surface area subject to that attrition is also being steadily diminished, with the result that the rate of solution in terms of percentage by weight or volume declines proportionately, till, by the time 95% of the carbonate has been dissolved, calcium is coming into solution at less than one eighth of the initial rate. If one assumes that dissolving conditions and the value of Δ remain constant till solution is complete, the life of any sized particle can be calculated; but it is clear that the protracted solution of the dwindling residual carbonate centres resulting from a normal application of limestone cannot provide soluble calcium at a useful rate. By way of illustrating this, the life period of the 10– and 20–mesh particles (under given conditions) is shown in Fig. 6, where it will be noticed in both cases that the final 12.5% of solution takes as long as the preceding 87.5%. This is due to the fact that a 50% reduction in diameter corresponds to an 87.5% reduction in the volume of the particles, and that the second 50% of diameter takes just as long to dissolve away as the first. Point A on the 10–mesh solution curve (Fig. 6) therefore represents the point where the 10–mesh particles have dwindled to 20–mesh size; and the remaining portion of the 10–mesh curve is in fact identical with the 20–mesh solution curve, except that its ordinate scale is reduced to one eighth.

CONCLUSIONS

The experimental results presented here, together with the analysis of previously published data, have established the fact that, under conditions of intimate admixture with a moist acid soil, carbonate particles of all the coarser sizes (> 80 mesh) representing some 50% to 80% of most commercial crushed limestones, undergo by solution an approximately equal rate of diameter reduction, so that, on the basis of some known or assumed rate of diameter reduction (Δ), it is possible to predict mathematically the progressive solution of the different sieve separates, their longevity, and their ability to maintain a supply of soluble calcium to the soil. The actual value of Δ depends, of course, on the type of limestone and on temperature and other soil conditions. The most practical outcome is that fineness of grinding exerts by far the greatest influence on rate of solution, differences due to hardness of the stone being small by comparison. Though not determined experimentally, it is obvious that the solution of the fine floury portions (< 150 mesh), present to the extent of 20% to 50% in all crushed limestones, must be very rapid indeed, both on account of their high specific surface and of that intenser dissolving action, revealed at the surface of the 80–150 mesh separates, which presumably affects all the finer particles. For all practical purposes these finer portions can be regarded as totally and immediately soluble when incorporated into a moist acid soil. One consequence of this is that the difference in solubility of coarsely and finely crushed agricultural limestones is occasioned only by their different contents of the gritty fractions, and is therefore less than might be supposed, when subject to any reasonable degree or period of solution. Thus the difference in 12 months' solution of "coarse" and "fine" commercial samples of the same stone would not amount to more than about 15%.

The steady and marked decline in the percentage rate of solution as the dissolving particles dwindle in size, suggests that an effective rate of supply of soluble calcium may only be expected from perhaps the first three-quarters of a dressing, unless that dressing is heavier than usual. This fact must doubtless be included among those responsible for the so-called "liming factor" which relates the results of laboratory tests to the field effects of limestone dressings.

It must be emphasized that the simple situation revealed here applies only to carbonate particles intimately mixed at modest rates with a moist and sufficiently acid soil. These are the conditions that enable calcium ions to be removed from the vicinity of the dissolving surface by diffusion and adsorption at a rate sufficient to maintain a steady dissolving power and a constant value of Δ. From observation on the solution of heavier applications (to be reported) and the progress of topdressing trials now under way, it would appear that a higher lime:soil ratio, such as would be occasioned by heavier application, indifferent admixture with the soil, or surface application, results in the early establishment of a degree of base saturation in the soil surrounding the particles which appreciably depresses the value of Δ. Since this effect is more rapidly established by the quick solution of the finer separates, the result is that under these conditions the finer particles lose much of their superiority in respect to rate of solution, a point that will be reported on more fully in due course.

Although the hypothesis of equal reduction herein established readily explains the diversity of the conclusions drawn by different workers on the relative solubilities of different-sized carbonate particles, it does not provide any escape from the fact that the *apparent* relative solubilities of fine and coarse, hard and soft limes, are inevitably governed by the degree of diameter reduction d to which their particles are submitted. Whether it be by the hardness of the stone, or the feeble dissolving power of the soil, or the shortness of the period of observation, if d be small, then differences will be maximal and, during the earliest stage of solution, may be exaggerated out of all proportion to their practical significance. On the other hand, if d be large, as in the case of long term comparisons of several years' duration, then differences become minimal and ultimately disappear altogether. In fact, as observed by Fippin[5] there is no *one* answer to this problem. It must remain a matter of judgement whether comparisons at the end of 3 months, 12 months, or several years are the most useful. This does not mean, however, that nothing useful can be done in the way of grading agricultural limestones in respect of their relative solubilities. But this is a question that can best be taken up when the influence of type of stone can also be taken into account. This will be dealt with in the third paper of this series.

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CORRIGENDUM

A corrigendum sheet is enclosed in this issue, for insertion at page 375, Volume 36, A (December 1954).

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